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Protomyces Comparative Genomics and Modulation of *Arabidopsis* Immunity

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Academic Dissertation

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Original publications

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Author´s contribution:

I. K.W. performed all experiments, K.W. and K.O., wrote the manuscript, all authors designed experiments, analyzed data, edited and approved the manuscript.

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III. K.W. participated in designing experiments, performed all the experiments and bioinformatics analyses. K.W. and K.O., wrote the manuscript.

Abbreviations

BAK	BRI1-associated receptor kinase
BIK	*Botrytis* -induced kinase
Botrytis	*Botrytis cinerea*
BR	Brassinosteroid
CAZyme	Carbohydrate activity enzyme
CERK1	Chitin elicitor receptor kinase 1
CFU	Colony forming unit
CK	Cytokinin
Col-0	Columbia-0 accession
CSEP	Candidate secreted effector protein
CSSP	Cysteine-rich small secreted protein
DAMP	Damage-associated molecular pattern
ET	Ethylene
ETI	Effector-triggered immunity
FLS2	FLAGELLIN-SENSITIVE 2
GA	Gibberellic acid
GYP	Glucose yeast-extract peptone medium
HR	Hypersensitive response
IAA	Indolic-3-acetic acid
IPyA	Indole-3-pyruvic acid
ITS	Internal transcribed spacer
JA	Jasmonic acid
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MDP	Microbe-derived phytohormone
MVC	Microbial volatile compounds
NAD	Nicotinamide adenine dinucleotide
NLR	Nucleotide-binding leucine-rich repeat receptor
NRP	Non-ribosomal peptide
ORFs	Open reading frames
OTU	Operational taxonomic unit
PAMP	Pathogen-associated molecular pattern
PB	Phyllosphere bacteria
PDA	Potato dextrose agar
PR	Pathogenesis-related gene
PRM	Phyllosphere resident microbe
PRR	Pattern recognition receptor
PTI	PAMP-triggered immunity
PTM	Post-translational modification
qPCR	Quantitative real-time PCR
RBOH	Respiratory burst oxidase homolog
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systematic acquired resistance
SC29	Protomyces sp. strain C29
SM	Secondary metabolite
SSP	Small secreted protein
TF	Transcription factor

Abstract

The plant phyllosphere environment offers a habitat for multiple kinds of microbes, including bacteria, fungi, yeast, etc. Microbes can be beneficial, pathogenic, or mostly neutral to plants. Increasingly the interaction patterns and related plant immunity signaling pathways against bacteria and filamentous fungi have been extensively studied. However, the interaction between plants and yeast or yeast-like fungi is largely unclear. Phyllosphere yeast-like fungi from wild *Arabidopsis* were isolated and characterized in this study. Around a hundred yeast isolates, including ascomycete *Protomyces* species, were identified and cultured. *Protomyces* species have been described as pathogens of plants in the Umbelliferae and Compositae families, however, with questionable phylogeny and little genomic information. We isolated and investigated the interaction of a strain *Protomyces* sp. SC29 (SC29) with *Arabidopsis*. SC29 can persist in the *Arabidopsis* phylloplane, and activate *Arabidopsis* immune responses with MAPKs (mitogen-activated protein kinase) activation and upregulation of salicylic acid signaling and camalexin biosynthesis marker genes. Additionally, indolic compounds produced by *Protomyces* species are able to activate plant auxin responses. The genomes of SC29 and all currently available *Protomyces* species were sequenced, assembled, and annotated. Comparative genomic analysis revealed genomic characters of SC29 related to adaptation to the phyllosphere environment. Genomic insights into the pathogenesis of *Protomyces* species were also discovered. The phylogenetic relationships of both the genus *Protomyces* and the subphylum Taphrinomycotina were re-constructed with genome-wide single-copy protein sequences. Small secreted proteins from the genomes of *Protomyces* spp. were analyzed as candidate effectors. Physiological, phylogenetic, and genomic evidence supported SC29 to be a novel species distinct from currently accepted *Protomyces* species. Thus, the study of SC29 and its interaction with *Arabidopsis* represents a new model system for the exploration of the genetics of plant interactions with phyllosphere resident yeasts.

1 Introduction

1.1 Microbial life in the phyllosphere environment

Microbes, including bacteria, fungi, and oomycetes have evolved strategies to survive in multiple environments, of which plants are one of the most abundant habitats with potential nutrients for colonization and propagation. Many microbes reside on the leaf surface (phyllosphere), around the root (rhizosphere) or inside tissues (endosphere) of one or more plant species. The term "phyllosphere" refers to the external surface environment of plants, and was first introduced in 1955 by F.T. Last (Last, 1955). Early studies focused on the impact of plant health and performance by phyllosphere resident microbes (PRMs). It has been proposed that resident microbes hinder disease development caused by pathogenic microbes. The disease inhibition effect might act through direct interactions such as antimicrobial compound production, and indirect interactions such as foliar nutrients competition, and altered host plant performance (Last and Deighton, 1965, Leben, 1965, Vacher et al., 2016). The tremendous complexity of PRMs was not realized until early 21st with the advance of finger-printing and meta-omics methods (Yang et al., 2001, Jumpponen and Jones, 2009). Subsequently, remarkable efforts were put into the field to understand the composition and function of PRMs. However, our understanding of the biology and ecology of phyllosphere resident yeast or yeast-like fungi remains largely empty.

1.1.1 The phyllosphere environment and PRM adaptation

Leaching of organic and inorganic nutrients from plant cells into the leaf surface (Tukey Jr, 1970, Leveau and Lindow, 2001) makes phyllosphere an appealing habitat for microbes. However, the phyllosphere is a nutrient-poor habitat that requires microbes to have specialized adaptation strategies to reside there. Additionally, PRMs must face multiple abiotic stresses, as well as biotic stresses from host plant and competitors. Abiotic stresses are significant to PRMs, including limited nutrient supply, exposure to UV and O_3, fluctuating temperature and humidity, among others (Vorholt, 2012). Biotic stresses are also harsh, PRMs must be resistant against antimicrobial compounds derived from both other microbe residents and host plant (Vorholt, 2012). Therefore, PRMs must have strategies to overcome the stresses and propagate in this hostile environment.

Phyllosphere bacteria (PB) have multiple adaptation strategies for the leaf surface environment. PB have metabolic adaptations such as the ability to assimilate methanol as carbon sources (Sy et al., 2005). PB can also manipulate cuticle permeability to increase leached sugars (Schreiber et al., 2005, Burch et al., 2014). Much evidence has shown that strains with deletions in pigment biosynthesis genes resulted in reduced survival under UV treatments (Jacobs et al., 2005). Additionally, DNA repair mechanisms are essential for *Pseudomonas* species to survive against UV on phyllosphere (Gunasekera and Sundin, 2006). The stress regulator protein PhyR, which regulates several oxidative response proteins, is essential for phyllosphere growth (Gourion et al., 2006). The production of phytohormones increases epiphytic fitness of some PB (Brandl and Lindow, 1998). As mentioned above, we have learned much about phyllosphere adaptations from multiple resident bacterial species. However, the adaptation mechanisms of phyllosphere yeasts or yeast-like fungi remain largely unknown, with limited genomic data and experimental confirmation.

1.1.2 Microbe-microbe interactions in the phylloplane

Microbes are always dealing with other neighboring microbes in multiple manners. The most famous microbe-microbe interaction case was the discovery of antibiotic penicillin produced by *Penicillium* mold against *Staphylococcus* strains (Fleming, 1929). Since then, many microbial interactions have been described. Increasingly evidence has shown fungal secondary metabolite gene clusters are activated upon direct contact with bacteria in mixed cultures, while the gene clusters are silent when cultured purely (Netzker et al., 2015). This indicates the complex nature of microbe-microbe interactions in plant phyllosphere.

Indeed, microbes have a wide range of secreted components with antifungal or antibacterial activity (Depoorter et al., 2016, Helfrich et al., 2018, Kemen et al., 2015, Mccormack et al., 1994, Suh et al., 2012, Melin et al., 2002, Golubev et al., 2008). Secreted effectors are described mostly in virulence roles against host plants, however, they may also be involved in microbe-microbe interactions (Suh et al., 2012, Melin et al., 2002). In addition to the growth inhibition effects, antibiotics produced by microbes have shown intermicrobial signaling functions. When sensing the antibiotics from a competitor, *Streptomyces* strains increase their own antibiotic production and suppress the competitor's production (Abrudan et al., 2015). Hub microbes are vital mediators for shaping the microbial community in the phyllosphere (Agler et al., 2016). Agler et al. demonstrated that the oomycete pathogen *Albugo* and basidiomycete yeast *Dioszegia* act as hubs to connect bacterial diversity and abundance in the *Arabidopsis* phyllosphere. The hub effect might be derived from direct inhibition and/or indirect interactions through host (Agler et al., 2016). The concept of hub microbes may offer new solutions for achieving plant disease resistance via manipulation of phyllosphere microbial communities.

1.1.3 Modes of microbial-host plant interactions

The roles of microbes in relation to plants can be beneficial, detrimental, or for most of the cases, neutral. Various evidence of plant growth-promoting phenotypes provide convincing arguments for the host beneficial effect of microbes. The molecular mechanisms of such beneficial interactions are being explored but are not fully understood. The manipulation of plant hormone networks has been proposed as a major mechanism. Bacteria and fungi produce phytohormones including auxins, CKs, GAs, and others. Altered growth phenotypes have been displayed by virtue of microbe-derived phytohormones (MDPs), suggesting the positive roles of MDPs. Manipulation of plant hormone signaling also contributes to growth promoting effects. For example, ACC (1-aminocyclopropane-1-carboxylate) deaminase from *Burkholderia* spp. can regulate ethylene levels to enhance plant growth (Onofre-Lemus et al., 2009). In addition, microbial volatile compounds (MVCs), such as 2,3-butanediol and acetoin, modulate plant hormone signaling for growth promotion as well as induced resistance (Tyagi et al., 2018, Ryu et al., 2003). Other mechanisms such as assisting mineral assimilation with siderophores (Sharma and Johri, 2003) and the beneficial effect of secondary metabolites (Esmaeel et al., 2018) have also been proposed.

The production of phytohormones by microbes can also be detrimental to plants. Plant hormones produced by pathogenic microbes mostly act as virulence factors, but the underlying mechanisms are not fully understood (Robert-Seilaniantz et al., 2011). The pathogen virulence mechanisms will

be further discussed later in section 1.2.1. Facing microbial challenge, even from potential pathogens, can have unexpected beneficial side effects. Since plants are suffering multiple biotic and abiotic stresses in their natural environment, a priming effect triggered by phyllosphere microbes may increase plant resistance and fitness to other stresses. The beneficial endophytes might become pathogenic when plants suffer stresses or undergo senescence (Delaye et al., 2013, Junker et al., 2012). Surprisingly, the presence of microbes in the phyllosphere, either beneficial or pathogenic, can increase plants fitness against herbivore stress (Saleem et al., 2017). Root bacterial communities have been demonstrated to protect plants against pathogens from other kingdoms and promote plant performance (Santhanam et al., 2015, Durán et al., 2018).

1.2 Overview of the plant immune system

Plants have no mobile cells to fight against invading pathogens, but have evolved multi-layer inducible immunity strategies to survive when facing pathogens. This type of immunity is also called innate immunity and all plant cells have capabilities for this immune response. Unlike in laboratory conditions, plants might continuously activate immunity systems in natural environments. Only a small portion of microbes can successfully invade plant cells and cause disease and/or eventually kill the plant. As the invader, microbes require weapons to attack; on the other hand, as the defender with low mobility, plants require strategies to protect themselves. An overview of microbial virulence mechanisms and plant defense strategies, mainly from plant-fungal and plant-bacterial interactions, is offered in the following paragraphs. The mechanisms of plant-yeast or yeast-like fungi interaction are less understood.

1.2.1 Plant immune system

Plant resistance can be broadly classified as preformed defenses and inducible defenses. Plant preformed defenses consists of preformed physical and chemical barriers that can inhibit most pathogens. Physical barriers, such as trichromes, cuticles, and cell walls, form the first layer of defense (**Fig. 1**) (Ziv et al., 2018, Aragón et al., 2017). Preformed chemicals present before microbial challenge, named as phytoanticipins, consist of secondary metabolites with antimicrobial properties that constitutively present (Vanetten et al., 1994). For instance, *Arabidopsis* phytoanticipin glucosinolate established plant resistance against the oomycete pathogen *Phytophthora brassicae*, together with the phytoalexin camalexin (Schlaeppi and Mauch, 2010). Preformed resistance prevents damage potentially caused by most microbes.

Plant inducible immunity consists of chemical metabolites and proteins, which act when plants recognize the presence of invading pathogens or potential pathogens. Plants can recognize the existence of microbial virulence factors and other conserved molecular patterns. Pathogen associated molecular patterns (PAMPs), such as fungal chitin and bacterial flagellin, refer to evolutionarily conserved pathogen molecules that elicit innate immunity (Zipfel and Felix, 2005). However, some similar defense-inducing molecules are also present in non-pathogenic microbes. Thus, the term microbe associated molecular patterns (MAMPs) broadens the concept of PAMPs (Ausubel, 2005). The immunity caused by this type of recognition is called pattern-triggered immunity

(PTI) (**Fig. 1**). Historically, PAMPs and DAMPs (damage associated molecular patterns) are thought to be widely distributed and highly conserved, yet, there are debates initiated from the cases which show PAMPs with narrow distributions among microbial species (Thomma et al., 2011).

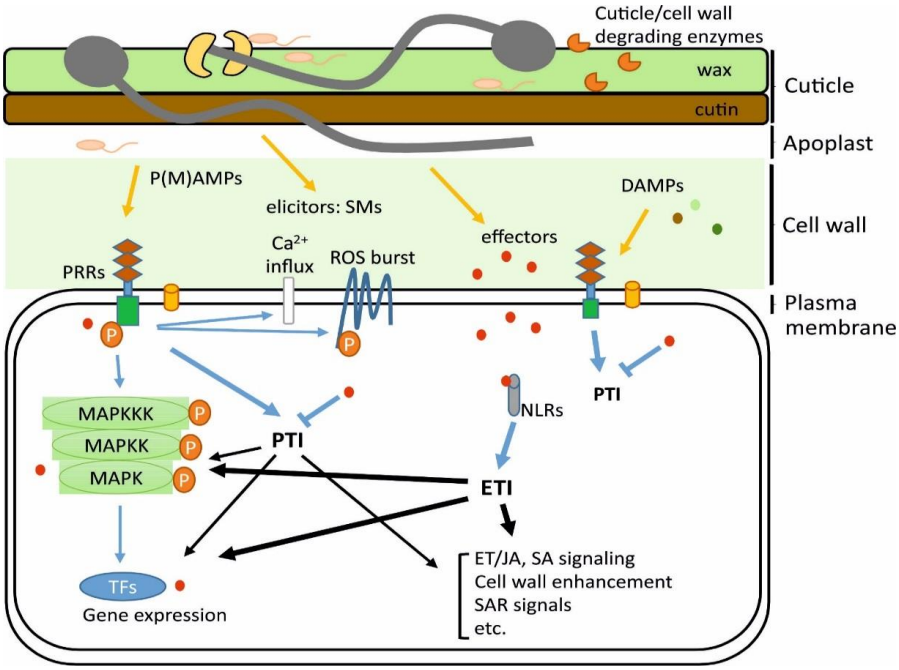

Figure 1. Overview of the battleground between pathogens and a plant cell. Pathogens apply cuticle/cell wall degrading enzymes to break down plant tissues at the external layers. Successful pathogens secrete effector proteins to overcome plant innate immunity. Plant cells can recognize the presence of an invading pathogen by extracellular transmembrane pattern recognition receptors (PRRs). Pathogen associated molecular patterns (PAMPs) or microbe associated molecular patterns (MAMPs) and damage associated molecular patterns (DAMPs) are perceived by PRRs, leading to PAMP triggered immunity (PTI). Plants also apply Resistance (R) proteins, which are nucleotide-binding leucine-rich repeat receptors (NLRs) to detect effector protein activity, leading to a stronger type of immunity than PTI, called effector triggered immunity (ETI). Both ETI and PTI result in immune responses such as ROS burst, Ca^{2+} influx, MAPK cascade activation, among others. This figure was based on Ziv et al. 2018 and Aragón et al. 2017.

DAMPs, such as fragmented cell wall components, released wax and cuticle components, can be detected by receptors located on plasma membrane. Other basic molecules participating in metabolism can also act as defense signals when leaking from damaged plant cells (**Fig. 1**). Extracellular ATP acts as a DAMP signal and is recognized by a lectin receptor kinase DORN1 (DOES NOT RESPOND TO NUCLEOTIDES) in *Arabidopsis* (Choi et al., 2014, Jeter et al., 2004). OGs (oligogalacturonides) can also trigger plant immunity response via plant receptor WAK1 (WALL-ASSOCIATED KINASE) (Denoux et al., 2008, Brutus et al., 2010, Decreux and Messiaen, 2005). Extracellular nicotinamide adenine dinucleotide (eNAD) has been shown the signaling function to improve resistance against biotic stress (Pétriacq et al., 2013). In addition, a recent study has identified a Lectin Receptor Kinase (LecRK) that binds to extracellular NAD^+ to trigger PTI in

Arabidopsis (Wang et al., 2017). Our knowledge of DAMPs and their associated receptors remains largely incomplete. The main components and signaling of plant inducible immunity are reviewed in the following sections.

1.2.1.1 Extracellular receptors

Plants deploy two major types of immune receptors: extracellular transmembrane pattern recognition receptors (PRRs) that perceive PAMPs to trigger PTI or basal immunity, and intracellular nucleotide-binding leucine-rich repeat receptor (NLR) that most often detect the activity of pathogen effector proteins to trigger effector triggered immunity (ETI). NLR receptors are subdivided into TNLs (TIR-domain nucleotide-binding leucine-rich repeat) and CNL (coiled-coil nucleotide-binding leucine-rich repeat) based on their N-terminus domains. PRRs respond to PAMPs or MAMPs, which are molecules that evolve slowly, such as bacterial flagellin (Hayashi et al., 2001, Felix et al., 1999) and fungal chitin (Shibuya and Minami, 2001). Recently, a bacterial fatty acid was shown to trigger immunity mediated by *Arabidopsis* receptor kinase LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION) (Kutschera et al., 2019).

Plant genomes possess a high number of receptor-like kinase and NLR genes. In the *Arabidopsis* genome, over 600 receptor-like kinase genes and about 150 NLR genes are predicted (Meyers et al., 2003, Shiu and Bleecker, 2003). The most well-characterized receptor model is "FLS2 (FLAGELLIN SENSITIVE2)-BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1)" flagellin PAMP receptor complex. The receptor-like kinase BAK1 and FLS2 form a complex after FLS2 binding with its ligand flagellin, thus being active for downstream signaling (Chinchilla et al., 2007, Heese et al., 2007). The receptor-like cytoplasmic kinase BIK1 (*BOTRYTIS*-INDUCED KINASE 1) was later identified as an essential component of FLS2-BAK1 complex (Lu et al., 2010). Following FLS2 activation, BIK1 was released from the receptor complex and directly phosphorylates the NADPH oxidase RBOHD (RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN D) to activate an extracellular ROS (reactive oxygen species) burst, in a Ca^{2+}-independent manner (Kadota et al., 2014, Li et al., 2014). It is noteworthy that BAK1 is a necessary component in multiple receptor signaling complexes, not only in innate immunity (Chinchilla et al., 2009).

In response to fungal pathogens, the cell wall component chitin is recognized by LysM (Lysine Motif) kinase receptor CERK1 (CHITIN ELICITOR RECEPTOR KINASE 1) in *Arabidopsis* and rice (Miya et al., 2007, Shimizu et al., 2010). As an essential co-receptor, CERK1 requires other kinase receptors/proteins for ligand-induced function, which is LYK5 in *Arabidopsis* (Cao et al., 2014), and CEBiP in rice (Shimizu et al., 2010). In addition to fungal chitin, CERK1 also mediates recognition of other fungal and bacterial PAMPs, such as peptidoglycan, lipopolysaccharide, β-glucan, which was recently reviewed (Desaki et al., 2019).

1.2.1.2 ROS and Ca^{2+} as early signals

Ca^{2+} is a critical signal for both intra- and inter-cellular signaling for many stress and developmental processes. The concentration of Ca^{2+} in cytoplast (less than μM) is much lower than that in apoplast and vacuole (about mM). Ca^{2+} flux in cytoplast is an early signal and is often coupled with a ROS burst in the apoplast when plant cells are challenged with stresses. Plant Ca^{2+} channels have been recently

reviewed (Demidchik et al., 2018). In potato, the increase of Ca^{2+} in the cytoplasm causes the activation of CDPKs (calcium dependent protein kinases), which directly phosphorylate RBOHs to induce a ROS burst (Kobayashi et al., 2007). However, how Ca^{2+} enters the cytoplasm of plant cells from the apoplast is unclear. A recent study demonstrated two cyclic nucleotide-gated channel proteins CNGC2 and CNGC4 that are required for PTI when Ca^{2+} supply is sufficient in *Arabidopsis*. When a PAMP signal is perceived, BIK1 phosphorylates and actives the Ca^{2+} channel where CNGC2 and CNGC4 proteins are assembled, leading to an increase in cytosolic calcium concentration (Tian et al., 2019).

ROS include singlet oxygen ($^1O_2$), superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH), among which H_2O_2 is most stable and important in redox signaling (Noctor et al., 2017). Inside plant cells, ROS are generated during normal photosynthesis in chloroplasts and during metabolic reactions in mitochondria and peroxisomes. In the extracellular space, ROS are produced mainly by respiratory burst oxidase homologs (RBOHs) and cell wall peroxidases during stresses and development (Wrzaczek et al., 2013, Waszczak et al., 2018). The apoplastic ROS burst directly strengthens cell walls by glycoprotein cross-linkage resulting in protection against further infection (Bradley et al., 1992).

Extracellular ROS assists to open Ca^{2+} influx channels. The ROS burst through transmembrane RBOHD requires Ca^{2+} binding with N-terminus EF hand motifs. Thus, it is always debatable which signal comes first. Some studies suggest that Ca^{2+} influx both precedes and depends on a ROS burst during stress signaling (Grant et al., 2000, Beneloujaephajri et al., 2013). Recently, a ROS-Ca^{2+} hub mechanism that self-amplifies the signal has been proposed, showing ROS and Ca^{2+} act in a coordinated manner (Demidchik and Shabala, 2018). Upon pathogen recognition, plant MAP4 Kinase, SIK1 (SALT INDUCIBLE KINASE 1), together with activated BIK1, phosphorylates RBOHD, leading to an extracellular ROS burst (Zhang et al., 2018). H_2O_2 acts as mobile signal that enters neighboring cells to spread defense signaling. Intracellular ROS signaling leads to downstream MAPK activation, interaction with phytohormones and TFs, and altered gene expression (Overmyer et al., 2018, Tuzet et al., 2018). ROS transmit signals to MAPK cascades by activating the *Arabidopsis* kinase, OXIDATIVE SIGNAL-INDUCER 1 (OXI1), which is required for MAPKs activation (Rentel et al., 2004). Activation of a MAPK cascade also leads to bursts of ROS and NO by participating in activating *RBOH* gene expression in tobacco (Asai et al., 2008).

1.2.1.3 Hormone signaling and hormone interactions

It has been long proposed that salicylic acid (SA) signaling is activated upon the recognition of biotrophic pathogens. ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4) function upstream of SA in PTI and effector triggered immunity (ETI) (Vlot et al., 2009), which is further discussed below. SA also positively regulates the expression of *EDS1* and *PAD4* genes (Vlot et al., 2009, Cui et al., 2017). SA is believed largely synthesized by ISOCHORISMATE SYNTHASE (ICS) and ISOCHORISMATE PYRUVATE LYASE (IPL) pathway from precursor chorismate (Vlot et al., 2009). However, since plants lack orthologs of IPLs, the full SA biosynthesis pathway remains unclear in plants. Recently, PBS3 (avrPPHB SUSCEPTIBLE 3) and EPS1 (ENHANCED PSEUDOMONAS SUSCEPTIBILITY 1) are proven to participate directly as key enzymes in the pathway, which completes

the SA biosynthesis mystery in plants (Rekhter et al., 2019). A group of mobile signals are responsible for systemic acquired resistance (SAR), including methyl salicylate (MeSA), dehydroabietinal (DA), glycerol-3-phosphate (G3P), azelaic acid (AzA), pepocolic acid (Pip) (Ádám et al., 2018, Klessig, 2012).

NPR1 (NON EXPRESSER OF PATHOGENESIS RELATED 1) is a master transcription cofactor that regulates SA-dependent SAR and SA-independent induced systemic resistance (ISR) (Withers and Dong, 2016). High concentrations of SA leads to dissociation of NPR1 oligomer complex (Tada et al., 2008). Monomer NPR1 moves to nucleus for binding to TGA (TGACG SEQUENCE-SPECIFIC BINDING PROTEIN) TF family members, leading to SA-responsive gene expression such as *PR-1* (Choi et al., 2010, Zhang et al., 2003). In addition, NPR1 is a direct SA receptor that functions as transcriptional coactivator upon binding with SA via copper metal (Wu et al., 2012). NPR3 and NPR4, paralogues of NPR1, are also SA receptors and mediate NPR1 degradation via an E3 ligase (Fu et al., 2012). A recent study showed that NPR3/NPR4 function as transcriptional co-repressors and NPR1 as a transcriptional co-activator, both of which bind TGA TF family members but function in a opposite manner (Ding et al., 2018).

Classically, SA mediates plant immunity against biotrophic and hemibiotrophic pathogens, while jasmonic acid (JA) and ethylene (ET) signaling fight against necrotrophic pathogens. The relation between these two basic forms of immune signaling is almost always antagonistic (Overmyer et al., 2018). However, with more evidence emerging, the network is far more complex than previously thought. Other abiotic stress and development hormones are also involved in biotic stress responses. For instance, ABA has been shown to be a negative defense regulator of immunity against necrotrophic pathogens (Liu et al., 2015). ABA is also suggested to be inhibitory to SA signaling by promoting NPR1 degradation (Ding et al., 2016). Gibberellic acid (GA) leads to elevated SA accumulation and the attenuation of JA signaling (Achard et al., 2003). Auxin leads to susceptibility (Navarro et al., 2006) and shows an antagonistic effect with SA. Brassinosteroid (BR) is involved in promoting plant growth and development, but also acts as negative and positive regulator in innate immunity (Nakashita et al., 2003, Choudhary et al., 2012). BR has been shown to stimulate JA biosynthesis and interact with other hormones, and thus, being a tradeoff regulator between defense and growth (Bajguz and Hayat, 2009, Choudhary et al., 2012). Cytokinins (CKs) promote plant immunity by SA signaling and NPR1/TGA3 (Choi et al., 2010). Many other interactions of phytohormones exist beyond those mentioned above, and have been discussed in the literature (Robert-Seilaniantz et al., 2007, Pieterse et al., 2009, Robert-Seilaniantz et al., 2011, Shigenaga et al., 2017, Overmyer et al., 2018). Indeed, hormone interactions is a complex network, which is necessary for plants to optimize adaptive responses to cope with highly dynamic combinations of multiple biotic and abiotic stresses.

1.2.1.4 MAPK cascades

Upon receiving upstream signals from receptors, such as hormones or ROS (Asai and Yoshioka, 2008), MAPK cascades transduce, integrate, and amplify signals into downstream components, such as TFs (Qiu et al., 2008, Li et al., 2012). MAPK cascades consist of MAPKKKs (MAP3Ks), which phosphorylate and activate MAPKKs (MAP2Ks), which act on MAPKs, which lead to downstream modification of transcription factors and finally reprograming of the transcriptome. In *Arabidopsis*, there are about

60 members of MAP3Ks, 10 members of MAP2Ks and 20 members of MAPKs (Hamel et al., 2006). However, only a few components of MAPK cascades have been functionally characterized.

Multiple danger signals are thought to converge into conserved MAPK cascades for defense and resistance. In *Arabidopsis*, the first full MAPK cascade (MAP3K1-MAP2K4/5-MAPK3/6) has been identified as this type of conserved MAPK cascade (Asai et al., 2002). Both MAPK3 and MAPK6 are reported to be involved in many signaling process, including PAMP (Asai et al., 2002), O_3 (Ahlfors et al., 2004), H_2O_2 (Teige et al., 2004), and ET (Yoo et al., 2008) signaling pathways, as well as osmotic shock (Droillard et al., 2002), camalexin biosynthesis (Ren et al., 2008) and in many developmental processes (Colcombet and Hirt, 2008, Xu and Zhang, 2015). Specifically, MAPK3 is involved in ABA signaling (Gudesblat et al., 2007), while MAPK6 participates in JA signaling (Takahashi et al., 2007). Functioning together with MAPK4, MAPK6 promotes SA biosynthesis and signaling. MAPK4 is a negative regulator of SA signaling and also regulates JA/ET signaling (Brodersen et al., 2006). Yet, how MAPK3/6 achieves downstream specificity upon a certain signal remains largely unclear, although several mechanisms have been proposed (Su et al., 2015, Jalmi and Sinha, 2015).

Most recently, a regulatory loop involving MAPK3/6 (MAPK3/6-WRKY33-pipecolic acid) has been identified to promote SAR in *Arabidopsis* (Wang et al., 2018). N-hydroxypipecolic acid (NHP), a derivative of pipecolic acid, functions together with SA as the vital mediator of SAR (Hartmann et al., 2018). Additionally, MAPK3/6 can act as latent components that effect defense priming, with the accumulation of mRNA and inactive MAPK3/6 proteins (Beckers et al., 2009). MAPK signaling is also believed to be vital in plant immunity against nonhost potential pathogens, which means preventing the infection by most microbes (He et al., 2006).

1.2.1.5 TF families

Transcription factors (TFs) are proteins that directly bind to the *cis*-acting elements of a gene promoter region, controlling the activation or repression of a gene expression. Others proteins, named transcriptional cofactors, can interact with TFs to manipulate gene expression. In the model plant *Arabidopsis*, around 2000 TF genes are encoded in the genome (Riechmann et al., 2000). TF families involved in plant stress responses are mainly ERF, WRKY, MYB, TGA and NAC families that have been nicely reviewed in the literature (Withers and Dong, 2017, Jiang et al., 2017, Wang et al., 2016, Dubos et al., 2010, Birkenbihl et al., 2018). Strikingly, upon recognition of pathogen PAMPs, some WRKY TF members quickly bind to over a thousand genes, some of which are TF genes, suggesting the existence of subregulatory networks of TFs (Birkenbihl et al., 2017).

In addition to transcription changes during stress response, modulation of translation efficiency also plays vital roles. Recently, Xu et al. demonstrated that the R-motif in mRNA determines the translation efficiency during PTI, suggesting the importance of global translational reprogramming (Xu et al., 2017). As discussed before, post-translational modifications (PTMs) ensure the rapid changes in multiple signaling pathways for plant stress responses and also for pathogens to achieve virulence (Withers and Dong, 2017).

1.2.1.6 The zig-zag model and spatial immunity model of plant immunity

The zig-zag model described multiple layers of plant immunity against pathogens in a co-evolutionary view (Jones and Dangl, 2006). Plants recognize microbial PAMPs or MAMPs to achieve PTI, as mentioned in 1.2.2. Successful pathogens overcome PTI by exploiting effector proteins, which leads to plant susceptibility. Plant NLR receptors detect pathogen effector protein activity, thus lead to another type of immunity called ETI. ETI acts much stronger than PTI and often leads to hypersensitive response (HR), which is a programmed cell death mechanism that prevents disease development and mediates resistance. Most HR is associated with the activation of NLR proteins. Jones and Dangl indicated that ETI is an accelerated and enlarged PTI (Jones and Dangl, 2006).

Both plant NLR receptors and pathogen effectors evolve rapidly through multiple mechanisms (Meyers et al., 2003, Mukhtar et al., 2011, Chisholm et al., 2006). The subcellular localization of NLR receptors are from cytoplasmic side of the plasma membrane to the cytosol and nucleus, but most commonly in the cytosol. Coordination of subcellular compartments might be important for full ETI resistance (Chiang and Coaker, 2015). Upon effector recognition, NLR protein opens and exchanges ADP for ATP and is then active to initiate downstream signaling. Multiple signaling processes are guarded by NLR proteins, which lead to activating ETI when effector activity is recognized. For instance, NLR SUMM2 protects MAPK cascade against *Pseudomonas* effector (Zhang et al., 2012).

The signaling events immediately downstream of NLR activation by effector proteins are largely unclear. Only few strong signaling components have been identified through forward genetic screens, indicating the ETI signaling pathways might be short or highly redundant (Chiang and Coaker, 2015). EDS1 and PAD4 are required for mediation of signaling from multiple TNL type NLRs (Parker et al., 1996). NON-RACE SPECIFIC DISEASE RESISTANCE (NDR1) is required for signaling by CNL type NLRs (Century et al., 1995). Some NLR proteins can directly interact with TFs, indicating that the ETI signaling pathway could be quite short. However, for those NLRs that are localized at the plasma membrane and remain there when activated, the ETI reprogramming might be indirect. This may function by NLRs recruiting TFs at plasma membrane, or by NLRs transmitting signals to other components, such as MAPKs, to TFs (Cui et al., 2015). In addition to interacting with TFs, a recent study shows that ETI can lead to high nuclear pore permeability to allow increased signaling for stress responses (Gu et al., 2016), which might explain the possibility of high level resistance and HR. Most recently, *Arabidopsis* NPR receptor ZAR1 (HOPZ-ACTIVATED RESISTANCE 1) was reported to form a pentangular oligomerization complex that might initiate cell death for HR, upon the recognition of *Xanthomonas* effector AvrAC (Wang et al., 2019b, Wang et al., 2019a, Dangl and Jones, 2019).

The zig-zag model describes plant immunity based on the type of danger signals. However, the distinction between PAMPs and effectors can be blurred (Thomma et al., 2011). Thus, Aranka and Matthieu proposed the spatial immunity model, which depicts plant immunity depending on the microbial recognition site (Van Der Burgh and Joosten, 2019). In this model, the danger signal that recognized extracellularly is called extracellular immunogenic pattern (ExIP), which includes MAMPs, PAMPs, DAMPs and extracellular effectors. Plant immunity triggered by ExIP, through cell surface receptors, is termed as extracellularly triggered immunity (ExTI). The danger signals that are recognized intracellularly are named intracellular immunogenic pattern (InIP), trigger immunity in

the plant called, intracellularly triggered immunity (InTI). This new model facilitates scientists in the classification of danger signals and in describing immune signaling (Van Der Burgh and Joosten, 2019).

The presence of yeasts or yeast-like fungi in the phyllosphere environment can also trigger plant immune responses. The foliar application of yeast extract and live yeast cells could elicit plant defense, including activation of multiple hormone signaling pathways (Lee et al., 2017, Buxdorf et al., 2013, Moon et al., 2015). Treatment of plants with cutinase triggers plant defenses, suggesting the phyllosphere microbes which secret cutin degrading enzymes can be perceived by plants (Chassot et al., 2007). However, our understanding is still limited because of the lack of a model system for the study of phyllosphere resident yeast on *Arabidopsis*.

1.2.2 Microbial weapons to infect plants

Necrotrophic and biotrophic pathogens employ distinct strategies to attack plants. Necrotrophic pathogens invade plants by killing plant tissues. They live and propagate based on dead plant material. Biotrophic pathogens attack plants without destroying cells, establishing a feeding relation with the living plant cells. They derive nutrients through, usually a specialized structure named haustoria. Armed with diverse types of weapons, necrotrophic and biotrophic pathogens accomplish different infection outcomes. Both the distinct and common weapons from necrotrophic and biotrophic pathogens are discussed here.

1.2.2.1 Degrading enzymes

Necrotrophic pathogens usually secrete several types of carbohydrate active enzymes (CAZymes) to break down plant tissues. CAZyme genes are abundant in the genomes of both necrotrophic and biotrophic fungal pathogens. However, necrotrophic pathogens seem to have a greater amount and diversity of genes encoding cell wall degrading enzymes in their genomes (Kubicek et al., 2014, Zhao et al., 2013). Biotrophic pathogens also utilize CAZymes to overcome barriers in plant tissues. However, biotrophic pathogens tend to infect only a few host cells to form nutrient-absorbing structures. They complete an infection cycle only within the infection site, rather than degrading whole plant tissues.

1.2.2.2 Effector proteins

The secretion of effector proteins are used by both necrotrophic and biotrophic pathogens. The genomes of pathogenic fungi usually contain hundreds of genes encoding candidate secreted effector proteins (CSEPs). Effector proteins are secreted in the apoplast and can either function there (apoplastic effectors) or be taken up into the host cytoplasm (cytoplasmic effectors) (Stergiopoulos and De Wit, 2009). For biotrophic fungal pathogens, effector genes are normally highly expressed in the specialized haustorial structures (Petre et al., 2014). Interestingly, haustoria-produced effectors have a common N-terminal Y/F/WxC motif, which is absent in non-haustorial fungal and oomycete effectors (Godfrey et al., 2010). Sequences of effector proteins share little similarity. However, a few common signatures of effector proteins have been characterized by genomic analysis, such as N-terminal Y/F/WxC motif in some fungi (Godfrey et al., 2010) and N-terminal RXRL-dEER motif in oomycetes (Rehmany et al., 2005). Normally, both RXLE and dEER motifs are necessary for *Phytophthora* effectors to transport into plant cells (Dou et al., 2008).

Effector proteins are typically thought to be species/lineage specific. However, some effector groups are widespread at the kingdom level, including fungal LysM effectors which contain the conserved LysM domain, but no other recognizable protein domains (Kombrink and Thomma, 2013, De Jonge et al., 2010). LysM effectors bind to peptidoglycan and chitin (Buist et al., 2008) to protect fungal hyphae against degradation by plant chitinase. More interestingly, LysM effectors actively bind self-chitin fragments to avoid the recognition by host receptors, thus prevent chitin-triggered immunity, which is a form of PTI (De Jonge et al., 2010). How fungal effectors enter plant cells has long been a mystery. Effector proteins with an N-terminal RXRL motif, from oomycetes and fungi, can enter plant cells by binding to phospholipid (phosphatidylinositol-3-phosphate, PI3P). This type of entry is independent of the presence of pathogens, and widespread in plant, animal, and human cells (Kale et al., 2010).

To suppress host defenses, fungal and bacterial pathogens employ effector proteins to disturb certain components of plant immunity. Effector proteins from both fungal and bacterial pathogens have diverse targets of plant immunity signaling, including receptor kinases (Xiang et al., 2011, Gimenez-Ibanez et al., 2009), protein kinases (Shan et al., 2008, Irieda et al., 2019, Feng et al., 2012, Zhang et al., 2010), MAPK cascades (Meng and Zhang, 2013, Zhang et al., 2007, Zhang et al., 2012), transcription factors (Sarris et al., 2015), histones (Arbibe et al., 2007), DNA (Bogdanove et al., 2010, Kay et al., 2007), small RNAs (Yin et al., 2019), microRNAs (Navarro et al., 2008), among others. It seems almost every step of innate immunity signaling pathways are targeted by pathogen effectors, which have varied localizations inside plant cells, such as nucleus (Petre et al., 2015, Arbibe et al., 2007, Sarris et al., 2015), mitochondria (Petre et al., 2015), and chloroplast (Petre et al., 2015). Bacterial type III effector proteins AvrPto and AvrPtoB intercept defense signaling upstream of MAPKKK (He et al., 2006) and later it has been shown that AvrPto targets the bacterial flagellin PAMP receptor FLS2 but not its coreceptor BAK1 (Xiang et al., 2011, Shan et al., 2008). The target of AvrPtoB is the LysM receptor kinase CERK1, which perceive fungal MAMPs in plants (Gimenez-Ibanez et al., 2009). Effectors can also manipulate plant hormone synthesis and signaling, for instance salicylic acid (SA) (Liu et al., 2014), auxin (Chen et al., 2004) and abscisic acid (ABA) (De Torres-Zabala et al., 2007), to promote susceptibility. SA biosynthesis is widely targeted by effectors from fungi, oomycetes, and bacteria (Kazan and Lyons, 2014, Liu et al., 2014). Auxin signaling is actively targeted by effectors for virulence, such as type III effector AvrBs3 from *Xanthomonas campestris* pv. *vesicatoria* and AvrRpt2 from *Pseudomonas syringae* (Robert-Seilaniantz et al., 2007).

Most known effector proteins function alone. However, an apoplastic decoy strategy was discovered. *Phytophthora* effector PsXEG1 is targeted and functionally blocked by soybean inhibitor. However, *Phytophthora* utilize a decoy effector, PsXEG1-like effector, which binds to soybean inhibitor with more affinity, thus to free effector PsXEG1 for virulence (Ma et al., 2017). In addition to targeting plant molecules, effector proteins also bind self-molecules to avoid the recognition or damage by plant cells. CfAvr4 specifically binds fungal chitin and prevents its degradation by plant-derived chitinase (Van Den Burg et al., 2006). Other examples of LysM effectors have already been mentioned above.

1.2.2.3 Fungal secondary metabolites

Fungal secondary metabolites contain a variety of low molecular bioactive compounds, including trichothecenes (Rocha et al., 2005), polyketides (Howard and Valent, 1996, Dalmais et al., 2011), siderophores (Haas et al., 2008), and terpenes (Cimmino et al., 2014), which might contribute to fungal virulence against host plants. Strikingly, fusicoccin, a carbotricyclic terpene from *Fusicoccum amygdali*, caused leaf wilt by opening stomata and disturbing water balance (Cimmino et al., 2014). Moreover, a recent study has shown a *Fusarium* NRP (fusaoctaxin A) facilitates cell-to-cell invasion and deletion of its biosynthesis gene cluster compromises *Fusarium* pathogenicity in wheat (Jia et al., 2019). The genes of secondary metabolites are usually organized as gene clusters in fungal genomes. Multiple tools have been developed for prediction and mapping of secondary metabolite gene clusters (Weber et al., 2015, Khaldi et al., 2010). Although the number of new discoveries of fungal secondary metabolite gene clusters are dramatically increasing, their virulence roles and regulatory mechanisms remain largely unclear, thus, require further intensive studies.

1.3 Fungal genomes offer insights into plant fungal interactions and evolution

Whole genome sequencing ideally offers all genetic information carried by an organism. The first complete genome sequencing of a living organism was finished in 1995, from the pathogenic bacterium *Haemophilus influenzae* (Fleischmann et al., 1995). The approach of assembling random pieces of DNA into a chromosome was applied (Fleischmann et al., 1995). One year later, the first genome of a eukaryotic organism *Saccharomyces cerevisiae* (baker's yeast) was published through a worldwide collaboration (Goffeau et al., 1996). Around 6,000 protein-coding genes were predicted, however, this raised a new challenge of elucidating gene functions (Goffeau et al., 1996). In the following years, the genomes of a dramatically increasing number of species were sequenced, offering a massive amount of genomic information. Notably, the Joint Genome Institute (JGI; https://jgi.doe.gov/) has lunched the 1000 fungal genomes project that aims to sequence 1000 fungal genomes and to better understand fungal diversity. Together with increasing genome announcements, online tools for analyzing genomic features are continuously developing. Pathogen infection processes can be depicted from representative genes, such as effector-like proteins, plant tissue degrading enzymes, secondary metabolites, hormone biosynthesis genes, and drug detoxification enzymes (Cissé et al., 2013, Kämper et al., 2006).

1.3.1 Candidate fungal effector proteins

Typically, effector prediction from a fungal genome is achieved by selecting cysteine-rich small secreted proteins (CSSPs) (Stergiopoulos and De Wit, 2009) from ORFs (open reading frames). However, the small secreted proteins (SSPs) with low cysteine content should also not be neglected. In the past years, online tools have been developed to predict effector candidates from fungal secretomes (Sperschneider et al., 2018a, Sperschneider et al., 2016); these methods were reviewed recently (Jones et al., 2018). In addition, effector subcellular localization is also predictable (Sperschneider et al., 2017, Sperschneider et al., 2018b). Normally hundreds of candidate effector proteins can be predicted in a pathogenic fungal genome. Analysis of effector genes suggested that

they are under selection pressure and thus evolving fast in bacteria (Baltrus et al., 2011), fungi (Poppe et al., 2015) and oomycetes (Raffaele et al., 2010). The possible functions of effector proteins can be anticipated by searching conserved motifs with HMMER software (http://hmmer.org/) using the Pfam database (Finn et al., 2015). The discovery of new conserved and functioning motifs might also possible since effector proteins are highly diverse and mostly species/lineage specific. However, experimentally testing candidate effector protein function is still necessary; however this is a labor intensive and time consuming process.

1.3.2 Secondary metabolites

Fungal secondary metabolites (SMs) are important factors determining pathogenicity and beneficial effect against plants (Pusztahelyi et al., 2015). Genomic analysis reveals a tremendous number of SM biosynthesis genes in fungal genomes (Kjærbølling et al., 2018, Helaly et al., 2018, Pusztahelyi et al., 2015), thus offering solutions to understand their potential roles. Fungal SM biosynthesis genes are usually clustered in chromosomes and can be explored by online tools such as antiSMASH (Weber et al., 2015) and SMURF (Khaldi et al., 2010). SM gene clusters of multiple species can be nicely compared. Several SM genes that are not necessarily located in clusters, such as phytohormone biosynthesis genes, could be searched by collecting known homolog sequences from other species and using local installations of the BLAST search software. The presence of hormone biosynthesis pathways offering clues of possible involvement microbe-derived hormones in fungal-plant interactions. Yet, a comprehensive approach combining genomic, genetic, and chemical evidence is required for validation of SM function.

1.3.3 Phylogenomics

Several molecular identification DNA markers have been developed and are widely used as fungal barcodes, such as the D1/D2 domain of the large subunit ribosomal DNA and the internal transcribed spacer (ITS) located between the rRNA small subunit and large subunits genes (Schoch et al., 2012, Fell et al., 2000). ITS is the most frequently applied barcode maker for species identification and phylogenetic studies in fungi, but with certain issues in some cases (Begerow et al., 2010, Schoch et al., 2012). Other nuclear DNA markers have been suggested as lineage specific secondary DNA markers, such as: actin, translation elongation factors, tubulin, among others. These might offer valuable alternatives, in addition to the ITS barcode, for gaining phylogenetic information in fungi (Blackwell, 2011, Stielow et al., 2015). However, the evolutionary information offered by single gene is limited and often controversial. Therefore, multiple gene sequences, or ultimately, genome-wide concatenated conserved nuclear gene sequences are preferable for the purpose of phylogenetic study. Single copy genes are unique and highly conserved across species, thus are preferable sequences for building phylogeny trees and have been applied for inferring phylogenetic relations widely (Schmitt et al., 2009, Wiegmann et al., 2009, Li et al., 2017). The concatenated sequence of multiple single copy protein sequences from nuclear genomes provides solid molecular evidence on fungal evolutionary diversity and species classification.

1.4 Current knowledge of the genus *Protomyces*

1.4.1 The definition of the genus *Protomyces*

Protomyces is a fungal genus, belonging to family Protomycetaceae, order Taphrinales, class Taphrinomycetes, subdivision Taphrinomycotina and division Ascomycota. Notably, genus *Protomyces* and its sister genus *Taphrina* share many similarities in their life cycle, pathogenicity strategy, etc. (Fonseca and Rodrigues, 2011, Kurtzman, 2011, Sjamsuridzal et al., 1997). All the currently described species in the genus *Protomyces* are plant pathogens. Kurtzman stated that all known *Protomyces* spp. have hosts in Apiaceae, Compositae, Umbelliferae and other plants (Kurtzman, 2011). This claim was supported by citation of Tubaki (Tubaki, 1957) and Reddy and Kramer (Reddy and Kramer, 1975). However, Apiaceae and Umbelliferae are accepted dual names for the same family (Turland et al., 2018), and no other host plants outside Umbelliferae or Asteraceae were described or mentioned for accepted *Protomyces* species (Tubaki, 1957, Reddy and Kramer, 1975, Kurtzman, 2011). Considering these facts together, all plant hosts of currently accepted *Protomyces* species are members of just two families, Umbelliferae (Apiaceae) and Asteraceae (Compositae). The typical disease symptoms caused by *Protomyces* species are galls, tumor, swelling or blisters on stems, leaves, petioles, flowers, or fruits on host plants. The ascospores can grow on artificial medium in a yeast-like manner without producing hyphae, which occurs only during infection on host plants. Conjugated spores from complementary mating types are infective. Ascogenous cells (chlamydospores) are formed during infection in host tissues. Ascospores develop in ascogenous cells and are released later either directly from ascogenous cells or from vesicles that germinate from ascogenous cells (Kurtzman et al., 2011). In the following, the studies are introduced about the seven most commonly studied *Protomyces* species that are available in yeast culture collections (**III**, Table S1).

P. gravidus causes stem gall disease of giant ragweed (*Ambrosia trifida*) and common ragweed (*Ambrosia artemisiifolia*). *P. gravidus* is first reported as *P. macrosporus* Unger in 1884 and then named as a new species in 1907 (Davis, 1907). This disease was widely distributed in lowland regions of Arkansas, USA in late the twentieth century (Cartwright and Templeton, 1988) and reported in Louisiana, USA in 1995 (Holcomb, 1995). Under natural conditions, the resting spores from stem gall required five months dormancy before releasing the differentiated ascospores (Cartwright and Templeton, 1988). Artificial infection of giant ragweed seedlings with *P. gravidus* caused gall symptoms from all tested plants in chambers. Because of the specificity of its pathogenicity, *P. gravidus* has been tested as a mycoherbicide of ragweed, but with several biological and practical limitations (Cartwright and Templeton, 1988).

P. inundatus is reported to cause disease on species of wild celery (*Apium* spp.), wild carrot (*Daucus* spp.) and water parsnip (*Sium* spp.), causing small blister symptoms. The life cycle was clarified by Valadon et al. (Valadon et al., 1962). Studies have shown that only the diploid cells of *P. inundatus*, which resulted from fusion of endospores of the opposite strains, can infect the plant host. The budding cells from unfused endospores are non-infective (Valadon et al., 1962). A resting period is required for ascogenous cells to germinate for *Protomyces* spp., while *P. inundatus* is an exception (Valadon et al., 1962).

P. macrosporus Unger is the first described and the type species in genus *Protomyces*, discovered by Unger (Unger, 1833). For *P. macrosporus,* many host plants in the Apiaceae (Umbelliferae) family have been described in Europe (https://bladmineerders.nl) and other locations worldwide. In agriculture, the seed production of coriander (*Coriandrum sativum*) is threatened by stem gall disease, which is caused by *P. macrosporus* (Khan and Parveen, 2018, Pavgi and Mukhopadhyay, 1972, Malhotra et al., 2016).

P. inouyei induces gall symptoms on stems of Oriental false hawksbeard (*Youngia japonica*) (Sugiyama et al., 2006, Tubaki, 1957). The haploid and diploid stages of *P. inouyei* have been described along with *P. lactucaedebilis* and *P. pachydermus* (Tubaki, 1957). Two group I introns were discovered in small-subunit rRNA in *P. inouyei* and *P. pachydermus*, supporting the occurrence of horizontal transfer of group I introns from plants to fungi (Nishida et al., 1998, Nishida and Sugiyama, 1995, Nishida et al., 1993).

P. lactucaedebilis is reported to be pathogenic on wild lettuce (*Lactuca debilis*), with symptoms of galls and swellings on stem, leaves, and buds (Reddy and Kramer, 1975). *P. pachydermus* has been found worldwide and causes disease on several plant genera in the Asteraceae family (Reddy and Kramer, 1975).

There are around 80 *Protomyces* spp. listed in Mycobank database (www.mycobank.org/) as legitimate names. However, only 10 species were accepted by Reddy and Kramer (Reddy and Kramer, 1975), while many others were rejected or unavailable. Recent studies described *Protomyces* species causing infections on wild plants (Bacigálová, 2008, Bacigálová et al., 2005), which are consistent with the defined host range established by Reddy and Kramer (Reddy and Kramer, 1975) and might be considered as accepted species although no molecular data was presented (Kurtzman, 2011). The classification of *Protomyces* is still far from satisfactory as most of the preceding taxonomic research largely depends on phenotypes. Practically, culture specimens are available for only the above mentioned 6 *Protomyces* spp. This was determined from a survey of 30 yeast culture collections in July 2019 (**III**, Table S1). Thus, more isolation and classification studies are necessary for a better understanding of this genus. Furthermore, as previously stated, more molecular data are needed for resolving the phylogenetic boundaries within and between the genus *Protomyces* and related genera in the Protomycetaceae family (Kurtzman, 2011).

1.4.2 Unresolved in phylogenetic relationships in the genus *Protomyces*

Members of the Ascomycete subphylum Taphrinomycotina, including the *Protomyces*, have many ancestral characteristics including similarities to Basidiomycetes, as such they are important for understanding fungal evolution and the evolution of pathogenicity (Sugiyama et al., 2006). The taxonomic position of *Protomyces* and its related genera has reminded uncertain since the first introduction of *P. macrosporus* (Unger, 1833). Research on the taxonomic position of the genus *Protomyces*, mainly during early the 20th century, up to 1975, was summarized by Reddy and Kamer (Reddy and Kramer, 1975). The classification in species level is mainly based the morphology and the localization of the ascogenous cells in host tissues, as well as the host phylogeny (Reddy and Kramer,

1975). Historically, morphology, cytology, physiology and pathogenicity of multiple (putative) *Protomyces* species were well-studied, but their classification still remains uncertain.

Reddy and Kramer (Reddy and Kramer, 1975) proposed that *Protomyces* together with other four genera (*Burenia*, *Protomycopsis*, *Taphridium*, and *Volkartia*) make up the family Protomycetaceae. *P. inundatus* has been previously proposed into the *Burenia* genus (Reddy and Kramer, 1975). However, It has been noted that genus and species demarcation in *Protomyces* and the related genera are unclear and likely incorrect and that species samples as well as molecular comparisons are needed (Kurtzman, 2011).

2 Aims of the study

The study of plant-microbe interactions is most advanced in the interactions between plants and bacteria, with well-characterized bacterial PAMPs and effector proteins as well as corresponding receptors and receptor-like protein kinases from plants. Our understanding in plant-fungal interactions is less advanced, especially in the field of plant-yeast interactions. The primary aim of this study is to isolate and characterize *Arabidopsis* associated yeasts in order to build an *Arabidopsis*-yeast interaction system that will allow us to advance our understanding of phyllosphere resident yeasts and plant immunity against yeasts.

Specific objectives:

1. Isolate and characterize of phyllosphere-resident yeast-like fungi from leaf surface of wild *Arabidopsis thaliana*.
2. Reveal the genomic features of yeast *Protomyces* sp. SC29 (SC29) as related to its residence on the leaf surface-, and explore the interaction patterns of SC29 and *Arabidopsis* from this interaction system.
3. Proposal of SC29 as a new *Protomyces* species and reevaluation of the genus *Protomyces* with genomic evidence.

3 Materials and methods

SM gene clusters from the genomes of *Protomyces* spp. were identified with the online tool antiSMASH fungal version. Gene synteny of *Protomyces* SM gene clusters were plotted in R using the genoPlotR package using genebank files of gene clusters from each species as input. Sequence comparison was conducted with BLASTn using DNA sequence of contigs. Gene annotations were added from genome annotation files (**II**).

The details of other material and methods can be found in attached publication and manuscripts, with a brief summary in the following tables.

Table 1. Material and methods used in this study.

Material and methods	Publication or manuscript
Activation of auxin response	**I, II**
Altered root hair assay	**II**
Carbon assimilation	**II**
Genome wide phylogeny analysis	**II, III**
Genomic assembly and annotation	**II**
Genomic DNA extraction	**I, II**
Genomic mining of key enzymes	**II, III**
Indolic compounds assay	**I, II**
Phylogeny tree with nuclear markers	**II, III**
Protein extraction, SDS-PAGE, Western blot	**II**
Protomyces spp. growth assay	**II**
RNA isolation and qPCR	**II**
Selection and analysis of effector candidates	**II**
Yeast (re-)isolation from *Arabidopsis* leaf	**I, II**
Yeast camalexin sensitivity test	**I**
Yeast culturing	**I, II**
Yeast identification	**I, II**
Yeast pre-treatment and Botrytis infection	**II**
Yeast temperature tolerance	**I**

Table 2. Genomic methods and software tools run online or locally in Linux or R (3.5.1) for genomic analysis and phylogeny.

Methods and software	Website
BLAST, BLASTp, tBLASTn	https://blast.ncbi.nlm.nih.gov/Blast.cgi
OrthoFinder	http://www.stevekellylab.com/software/orthofinder
Guidance2	http://guidance.tau.ac.il/ver2/

FASconCAT_v1.0	https://www.zfmk.de/en/research/research-centres-and-groups/fasconcat
RAxML	https://cme.h-its.org/exelixis/software.html
iTOL	https://itol.embl.de/
ANI AAI calculator	http://enve-omics.ce.gatech.edu/
SignalP	http://www.cbs.dtu.dk/services/SignalP/
OrthoVenn	http://www.bioinfogenome.net/OrthoVenn/
HMMER	http://hmmer.org/
dbCAN	http://csbl.bmb.uga.edu/dbCAN/
PHI base	http://www.phi-base.org/
Badirate	http://www.ub.edu/softevol/badirate/
genoPlotR	http://genoplotr.r-forge.r-project.org/
ClustalX2	http://www.clustal.org/

Table 3. Defense related gene markers used for qPCR in Manuscript **II**.

Gene abbreviation	Gene name	Marker for	AGI code
ARR15	*RESPONSE REGULATOR 15*	Cytokinin signaling	At1g74890
CYP71a13	*CYTOCHROME P450, FAMILY 71, SUBFAMILY A, POLYPEPTIDE 13*	Camalexin	At2g30770
IAA7	*INDOLE-3-ACETIC ACID 7*	Auxin signaling	At3g23050
JAZ1	*JASMONATE-ZIM-DOMAIN PROTEIN 1*	Early JA signaling	At1g19180
NCED5	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 5*	ABA Biosynthesis	At1g30100
ODX/DIN11	*2-OXOACID-DEPENDENT DIOXYGENASE / DARK INDUCIBLE 11*	SA signaling	At3g49620
PAD3	*PHYTOALEXIN DEFICIENT 3*	Camalexin	At3g26830
PDF1.1	*PLANT DEFENSIN 1.1*	JA signaling	At1g75830
PDF1.2	*PLANT DEFENSIN 1.2*	JA signaling	At5g44420
PR-1	*PATHOGENESIS-RELATED GENE 1*	Late SA signaling	At2g14610
RAP2.6	*RELATED TO AP2 6*	JA signaling	At1g43160
SID2	*SALICYLIC ACID INDUCTION DEFICIENT 2*	SA biosynthesis	At1g74710
TIP41	*AP42 INTERACTING PROTEIN OF 41 KDA*	Reference gene	At4g34270
YLS8	*YELLOW-LEAF-SPECIFIC GENE 8*	Reference gene	At5g08290
PP2AA3	*PROTEIN PHOSPHATASE 2A SUBUNIT A3*	Reference gene	At1g13320

4 Results and discussion

4.1 Isolation and characterization of phyllosphere-resident yeast-like fungi

4.1.1 Collection of yeast strains

Arabidopsis rosettes were collected from three different sites (Kivikko, Kulosaari, and Mustikkamaa) at three different time points in Helsinki, Finland (**I**, Table 1). Dilutions of leaf wash solutions were plated on 0.2x PDA plates until colonies emerged under culture conditions that promoted yeast growth. Single yeast-like colonies were then collected for pure culture. Hundreds of strains were purified and yeasts were selected: in total around 6% ascomycetes and 94% basidiomycetes (**I**, Figure 2). Metagenomics with barcode markers would be an alternative approach to reveal the resident yeast community composition including uncultivable strains. Isolation method was chosen in this study because of the isolates it provided, which allows further study with selected strains. It is interesting to note that the ratio of ascomycete isolates is less than those isolated from tropical and warm regions (Limtong and Kaewwichian, 2015, Sláviková et al., 2009, De Azeredo et al., 2010). Several cold-adapted yeast strains are present in our isolation, with active growth at 8°C (**I**, Figure 6). Whether the ratio of ascomycete and basidiomycete species in the phyllosphere is related to the latitude or temperature is unclear, but it may be worthwhile to analyze this by performing more collections from multiple regions.

4.1.2 Identification and phylogeny with gene markers

The yeast strains isolated from the *Arabidopsis* leaf were identified by a combined molecular approach. Internal transcribed spacer (ITS) fragment sizes and restriction enzymes patterns (RFLPs: restriction fragment length polymorphisms) were used to assign strains into operational taxonomic units (OTUs). In total, 33 OTUs were listed in this collection (**I**, Table 2). The full ITS region (ITS1, 5.8 S, and ITS2) of representative strain(s) from each OTU were sequenced. As a classification result, 95 yeast strains were placed into 23 species from 9 genera (**I**, Table 2). Basidiomycetes dominate *Arabidopsis* phyllosphere yeast community, with over 90% of total strains. However, three OTUs of ascomycetes (*Protomyces* and *Taphrina* spp.) were identified, which are genera previously described as plant pathogens. The ITS marker was described as a fungal identification marker with easy procedures and high efficiency (Nilsson et al., 2014, Schoch et al., 2012). Here we presented a combined approach with restriction enzymes patterns and ITS sequencing that benefits classification and identification of culture-dependent microbial work.

In order to define the phylogenetic relationships within the genus *Protomyces*, we utilized the full ITS and D1/D2 region of large subunits of ribosomal DNA for the construction of phylogeny trees. However, the true diversity and phylogeny are not clearly resolved by ITS or D1/D2 sequences (**II**, Figure 1, a, b), which are highly conserved and underestimate the diversity among *Protomyces* spp. This has been previously observed in *Taphrina*, a sister genus of *Protomyces*, which has similar lifestyles, morphology and virulence strategies (Fonseca and Rodrigues, 2011). We were able to resolve the phylogeny of *Protomyces* species using whole genome data (**II**, Figure 1, c). However, in order to find out additional single gene DNA markers for rapid identification of *Protomyces* species, we investigated other nuclear gene makers; *ACT1*, *TUB2*, *RPB1*, *RPB2*, and *TEF1* (Stielow et al., 2015).

DNA sequences of these markers were extracted from *Protomyces* spp. genome annotations with *Schizosaccharomyces* sequences as query. Phylogenetic trees built with *ACT1* (**III**, Figure S2) displayed the closest branching pattern compared to genome-wide phylogeny tree (**II**, Figure 1, c). Therefore, *ACT1* is proposed as the most accurate gene marker for *Protomyces* spp. phylogeny in this study. The other four markers *TUB2*, *RPB1*, *RPB2*, and *TEF1* also exhibit clear species resolution but less accurate tree architectures. All of the five markers showed highly similar phylogenies for *Taphrina* and *Schizosaccharomyces* spp. Additionally, a phylogenetic tree with concatenated sequences from all five markers was provided (**III**, Figure S2), producing tree architectures similar with those of the*TUB2*, *RPB1*, *RPB2*, and *TEF1* single gene trees. Therefore, we conclude that the DNA sequence of the *ACT1* gene is favorable to rapidly explore phylogenetic relationships and identify species in genus *Protomyces*.

4.1.3 Camalexin sensitivity

Camalexin is an important phytoalexin that inhibits microbes probably by disrupting microbial cell membranes (Rogers et al., 1996). Several *Arabidopsis* pathogens are sensitive to camalexin (Thomma et al., 1999). In this study, most of isolated yeast strains are camalexin tolerant, while *Protomyces* and *Taphrina* are sensitive to 15 μg/ml camalexin in liquid culture. Non-pathogenic yeasts were able to tolerate growth in the presence of camalexin (**I**, Figure 5), suggesting they have the ability to degrade or remove toxins from their cells. For the potential pathogens that are sensitive to camalexin (**I**, Figure 5), they either manipulate plant immunity to survive or have their growth inhibited by the host plant immune system. Furthermore, the presence of *Protomyces* sp. SC29 on *Arabidopsis* can activate camalexin biosynthesis gene expression (II, Figure 2, d). The possible explanation could be either SC29 triggers plant immunity to inhibit other microbe competitors, or SC29 is recognized as a potential pathogen and its growth is limited by *Arabidopsis*.

4.2 Protomyces strain SC29 and its interaction with *Arabidopsis*

4.2.1 SC29 persists on the *Arabidopsis* leaf surface

Since SC29 was isolated from leaf surface of *Arabidopsis*, we were keen to understand whether SC29 could survive in the *Arabidopsis* phylloplane or if perhaps SC29 just landed by chance and was only transiently present. We performed growth assays with SC29 and its close species *P. inouyei* on the *Arabidopsis* leaf surface, with plants grown both in soil and sterile MS medium growth conditions. The colony forming units (CFUs) of *P. inouyei* dropped down rapidly, which was similar to the behavior of SC29 growing on foil surface, the negative control environment. In contrast, the CFUs of SC29 remained at the inoculation level at both three-day and ten-day time-points (**II**, Figure 2, a). This indicated SC29 might be adapted to the *Arabidopsis* phyllosphere environment, while *P. inouyei* was not adapted and rapidly died. This indicated SC29 is better adapted to *Arabidopsis* leaf surface, which did not occur with its closest relative *P. inouyei*.

We infected *Arabidopsis* with SC29 with different temperature conditions (8 °C and 23 °C), different infection methods (dropping and spraying) in growth chamber, and with overwinter period under outdoor field conditions (**II**, Table, S3). No visible disease-like symptom were observed in infected

plants. However, *Protomyces* persisted overwinter and could be re-isolated from field infected plants (paper II). Further, *Protomyces* was found on *Arabidopsis* in multiple sites and countries (**I**, Table 1; **II**, Figure S2; and (Agler et al., 2016), suggesting *Protomyces* has a real interaction with *Arabidopsis* as a host.

4.2.2 SC29 leads to decreased disease development

Increased resistance and activated immune signaling pathways have been observed from treating *Arabidopsis* with autoclaved cell suspensions of *S. cerevisiae* (Raacke et al., 2006) or plants treated with protein-depleted yeast extract (Moon et al., 2015). Cell wall carbohydrates from yeast cells may act as MAMPs that are recognized by the plant. We tested whether SC29 treatment could activate plant immunity and thus increase plant resistance against disease. Lesion diameters of *Botrytis cinerea* drop infections were used as a measure of disease development on plants either mock treated or sprayed with live or dead-SC29 cell suspensions. Intriguingly, *Arabidopsis* pre-treated with both live-SC29 and dead-SC29 exhibited less disease development, compared to *Arabidopsis* pre-treated with water (**II**, Figure 2, b). Live-SC29 pre-treated *Arabidopsis* showed smaller lesion diameter than dead SC29 pre-treated *Arabidopsis* (**II**, Figure 2, b). In addition, we demonstrated that SC29 has no direct antagonistic effect against *Botrytis* growth on GYP plates (**II**, Figure S4). These results suggested that treatment with SC29 activates plant immunity to protect *Arabidopsis* when facing *Botrytis* infection. Killed-SC29, likely representing a mixture molecules including PAMPs, activated *Arabidopsis* resistance, but a stronger induced resistance was noticed with live SC29 treatment. Either active processes in live cells are required, or some of the resistance-inducing molecules are destroyed by autoclaving.

4.2.3 MAPK3 and MAPK6 activation

Among the early immunity signaling events, MAPK cascade is believed a signal integration and amplification mechanism that initiates immunity responses against multiple stresses. To test the early signal events upon SC29 treatment, western blot with anti-TEpY antibody against active phosphorylated form of MAPKs was implemented to test if MAPK3/6 (MAPK3 and MAPK6) are involved in immunity activated by SC29. Both MAPK3 and MAPK6 are activated when *Arabidopsis* plants were treated with live- and dead-SC29 at 30 min and 60 min post pre-treatment (**II**, Figure 2, c). This supported the proposal that unknown SC29 PAMP(s) are recognized by *Arabidopsis* PRR(s), which leads to downstream immune signaling including MAPK3/6 activation.

However, no significantly stronger activation of MAPK3/6 was observed for SC29 pre-treated *Arabidopsis* after *Botrytis* infection, compared to water pre-treated plants (**II**, Figure 2, c). This might derived from that fact that *Botrytis* induced immunity response is so strong that it masks the SC29 primed immunity assayed at the level of MAPK activation. Alternatively, MAPK3/6 independent immune signaling, such as MAPK3/6 independent camalexin biosynthesis involving WRKY33 (Qiu et al., 2008), is also possible for SC29-triggered resistance against *Botrytis*.

4.2.4 Camalexin biosynthesis and salicylic acid signaling

Plants employ multiple and interacting signaling pathways to achieve immunity. We performed qPCR with multiple defense signaling markers to investigate the SC29-activated signaling pathways. The

camalexin biosynthesis gene *PAD3* was upregulated in both live- and dead-SC29 treated plants at 72 hpt (hour post pre-treatment). Two camalexin biosynthesis genes, *CYP71a13* and *PAD3,* were upregulated only in live-SC29 treated plants at 72 hpi (hour post infection), but not at 24 hpi (**II**, Figure 2, c). These results suggest that camalexin biosynthesis was involved in SC29-activated immunity. In fact, only a few PAMPs can induce camalexin biosynthesis in *Arabidopsis*, including peptidoglycans and Nep1-like proteins, but not flg22 or OGs (Meng and Zhang, 2013). Here we reported that live SC29 induced higher expression of camalexin biosynthesis genes, which are possibly upregulated also by SC29 PAMPs.

In addition, the SA signaling marker *PR1* was upregulated in live-SC29 treated plants at 72 hpi (**II**, Figure 2, c), suggesting that SA signaling also participated in SC29-activated immunity. JA signaling may participate in dead-SC29 triggered immunity as seen from upregulation of *PDF1.1* in dead-SC29 treated plants 24 hpi (**II**, Figure 2, c). Markers of other signaling pathways, including auxin, cytokinin, and ABA were not altered in expression level upon SC29 treatment (**II**, Figure S5). To conclude, SC29 can partially induce *Arabidopsis* resistance with MAPK3/6 activation and possible camalexin biosynthesis and JA signaling pathway marker activation. Yet, full induced resistance requires live SC29, with MAPK3/6 activation and transcriptional activation of markers for camalexin production and SA signaling.

4.2.5 Yeast auxin production and its possible roles

Microbe-derived phytohormones are believed to play multiple roles against plants (Jameson, 2000, Spaepen and Vanderleyden, 2011, Vadassery et al., 2008). Auxin is a main growth regulator, but also participates in modulating plant immunity (Spaepen and Vanderleyden, 2011). We tested indolic compound production, including auxin, by representative strains of each genus from our yeast isolation work. Among the tested strains, eight showed indolic compound production in GYP liquid media, most of which exhibited higher accumulation when the media was supplemented with additional tryptophan (**I**, Figure 3), which is a precursor of auxin biosynthesis. Strikingly, three strains (*Leucosporidium* sp. OTU26, *Microbotryozyma* sp. OTU28 and *Leucosporidium* sp. OTU27) displayed indolic compound production in tryptophan-free minimal media (**I**, Figure 3), suggesting the existence of possible tryptophan-independent synthesis pathways, or that these species have a higher level of *de novo* tryptophan biosynthesis. These strains are potential resources for studying tryptophan-independent auxin synthesis pathways. Additionally, all *Protomyces* spp. in this study produce indolic compounds in GYP media. The level of production was variable among species. However, significantly higher indolic compound production was observed for all *Protomyces* spp. when GYP media was supplemented with additional tryptophan (**II**, Figure 4, a). The enhanced production confirmed the precursor role of tryptophan in *Protomyces* spp. indolic compound synthesis.

The observed indolic compound production shown in previous section suggests these species may produce auxin. To confirm the auxin activity against plants, we treated *Arabidopsis* DR5::GUS (a reporter line for auxin response) with yeast culture filtrates. GUS activity induced by yeast culture filtrates (blue color of plant tissue after staining) were observed, especially from *Taphrina* sp. OTU3, *Protomyces* sp. OTU1, and *Dioszegia* sp. OTU23 (**I**, Figure 4; Figure S1). This observation strongly supports that yeast-derived indolic compounds have auxin activity *in planta*. Yet, the auxin activity

and indolic compound content of culture filtrates did not correlate well, suggesting the auxin-active content of total indolic compounds varies among different OTUs. To understand if *Protomyces* species-derived indolic compounds are active against plants, we examined the phenotypes of *Arabidopsis* auxin-response reporter line DR5:GUS and Col-0 root hair when treated with *Protomyces* spp. liquid culture filtrates. GUS activities were detected from DR5:GUS lines treated by all *Protomyces* spp. culture filtrates (**II**, Figure 4, b). Similarly, enhanced root hair phenotypes were observed from *Arabidopsis* Col-0 treated with all *Protomyces* culture filtrates (**II**, Figure 4, c). However, again the production of indolic compounds is not linearly related with the degree of GUS staining, neither with the degree of root hair induction. *Protomyces* species-derived auxins can activate plant response in a similar manner with plant auxins. Whether auxins are produced by SC29 while residing on *Arabidopsis* phylloplane remains unknown.

An intact IAA biosynthesis pathway (IPyA pathway) was found in all seven *Protomyces* spp., with genes of the key enzymes tryptophan aminotransferase, IPyA decarboxylase, and IAAld dehydrogenase present in their genomes **(II**, Table S5). We also searched for other proposed microbial IAA biosynthesis pathways but homologs representing the full pathway were not found (**II**, Table S5). Taken together with 4.2.5, we concluded that *Protomyces* spp. have IPyA IAA biosynthesis pathway, and can produced auxin or auxin-like indolic compounds that lead to the activation of *Arabidopsis* auxin responses.

4.3 *Protomyces* spp. genome shows insight for interaction and adaptation

4.3.1 *Protomyces* genomic characteristics

We sequenced the genomes of seven *Protomyces* species, including six well-described species and one recently isolated strain (SC29). All *Protomyces* spp. have small genome sizes (11.5-14.1 Mb) with 50.9-52.8% GC content. The annotated genes in *Protomyces* spp. genomes are between 5500 to 6300 genes. Genomes completeness scores are relatively high and similar in all species, ranging from 85% to 88% (**II**, Table 1), which indicate good quality draft genome assemblies. Sequencing raw data, genome assemblies, and annotations were submitted to genebank, or otherwise made publicly available, with accession numbers listed in (**II**, Table 1).

All carbohydrate enzyme classes and associated enzyme modules currently covered by CAZy database were discovered. Carbohydrate active enzymes (CAZymes) in *Protomyces* spp. were found in class auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyltransferases (GT) and polysaccharide lyases (PL). All the genomes of *Protomyces* spp. have lower amount of CAZymes (Zhao et al., 2013) and similar enzyme class composition, which is consistent with their biotrophic pathogen lifestyles. The number of CAZymes in all classes were listed in (**II**, File, S7). By experimental tests, the carbon assimilation patterns of *Protomyces* spp. are variable. We examined the carbon assimilation enzyme content in the genomes of these species and tried to correlate this with carbon utilization patterns. However, the enzymes involved in assimilation of some carbon sources were also present is species that are unable to utilize the carbon source (**III**, Figure 3). This suggested that other factors, such as DNA and histone

modification, translation, and post translation modification, might be responsible for disparate assimilation patterns, as has been previously suggested (Riley et al., 2016). Another explanation could be that the enzymes are present but play other fundamental roles rather than catalyzing carbons as energy sources.

Histidine hybrid kinases (HHKs) are important sensory kinases. In the genomes of *Protomyces* species, most of the HHKs groups were found (II, Table S6). Most interestingly, SC29 has significant expansion in group I HHKs, which have an undetermined function. However, a slight loss of virulence was seen when a group I HKK was mutated in rice blast fungal (Jacob et al., 2014). Further investigation revealed two group I HHKs genes in SC29 are adjacent to other HHKs, indicating the possible mechanism of creating new signaling components by gene duplication and mutation. Group III HHKs are found as two copies in some *Protomyces* spp., while they are normally present as single copy in other pathogenic fungi (Defosse et al., 2015). Moreover, Pmac has a dual HHK adjacent to a type XI HHK; dual HHKs have previously been found only in the Basidiomycota. Taken together, these data suggested that HHKs in *Protomyces* spp. are actively evolving and SC29 has unique HHKs futures among genus *Protomyces*. In addition, the dual HHK may be an example of an ancestral basidiomycete characteristic. *Protomyces* spp. also possess other basidiomycete characteristics. All the genomes of *Protomyces* spp. in this study have over 50% GC content, which consistent with basidiomycetous yeasts but not ascomycetous yeasts (Sugiyama et al., 1996). *Protomyces* spp. have an enteroblastic budding pattern, which is similar to basidiomycetous yeasts but different from the holoblastic budding seen in ascomycetous yeasts (Von Arx and Weijman, 1979, Sugiyama et al., 1996). Moreover, Q-10 is the major ubiquinone system in *Protomyces* and *Taphrina* spp., which is a basidiomycetous yeast character (Sugiyama et al., 2006, Sugiyama et al., 1996). In conclusion, we presented new genomic evidence, the dual dual HHK gene, in genus *Protomyces*, showing that genus *Protomyces* and subphylum Taphrinomycotina have ancestral characters which similar to basidiomycetous yeasts.

Genomic evidence suggests that SC29 might be adapted to live in the phyllosphere of *Arabidopsis*. Gene ontology (GO) enrichment of DNA repair genes was found in SC29 when compared to a clade consisting of Pino, Plac and Ppac, indicating a better adaptation ability for UV and ROS stress that commonly exist in the phyllosphere environment. On the other hand, invasive filamentous growth genes were noticed as gene loss in SC29, indicating the possibility of decreased pathogenicity, compared to Pino, Plac and Ppac. The cutinase transcription factor 1 family was expanded in SC29 specifically, which may be required for fungal cutinase induction and thus better utilization of this unique carbon sources found in the phyllosphere.

4.3.2 Candidate effector proteins

SSPs, including CSSPs, are regarded as candidates of effector proteins. The genomes of *Protomyces* spp. possess over a thousand SSPs and around 600 CSSPs (**II**, Table S4). The massive amount of effector candidates might function as important tools of plant-microbe and microbe-microbe interactions. Generally, all *Protomyces* spp. have similar SSPs features and higher cysteine content and shorter protein length than those of *T. deformans* (**II**, Figure S6). Positive hits of fungal virulence proteins and effector proteins in PHIbase were discovered from *Protomyces* spp. genomes (**II**,

Supplementary file S3), indicating the potential active roles of *Protomyces* spp. SSPs against plants. Despite of the general similarity of SSPs features among *Protomyces* spp., we noticed that SC29 has distinct characters compared to its closest clade (Pino, Plac and Ppac). The three species in that clade share 283 SSP orthologous clusters, while SC29 shares only 24, 40, 22 SSP orthologous clusters with any two of Pino, Plac and Ppac, respectively (**II**, Figure 3). This indicated that SC29 has disparate repertoire of effector candidates to the clade composed of Pino, Plac and Ppac. The plant hosts of SC29 as a pathogen are unclear, but we speculate the host range of SC29 is largely differ from Pino, Plac or Ppac.

By performing hmmscan with Pfam database, we discovered that *Protomyces* spp. genomes lack effector candidates containing LysM domains (**II**, Supplementary file S2). LysM domain effectors are commonly present in fungal effectors to mask chitin from recognition or degradation by plants. Yeast cell walls are generally composed of chitin, beta-glucans, and mannoproteins (Perez-Garcia et al., 2011). *Candida albicans* is a human pathogenic yeast, which lacks LysM effectors. The ability of cell wall components other than chitin from *C. albicans* to trigger immune cell activation in humans has been shown (Perez-Garcia et al., 2011). The lack of LysM effector in *Protomyces* suggests that chitin is less important in their interaction with plants. However, other lectin domains are present among *Protomyces* spp. effector candidates (**II**, Supplementary file S2). Maize uses secreted proteins (AFP1 and AFP2) with antifungal activity that bind to pathogen hyphal mannose. The maize pathogen *Ustilago maydis* responds with the Rsp3 lectin domain effector that binds to AFP1 and AFP2 to block their antifungal activity. The presence of lectin domain effectors in *Protomyces* spp. suggests mannose or other unique carbohydrates found in the yeast cell wall might be the PAMP that is protected by these lectin effectors.

4.3.3 Secondary metabolite gene clusters

Fungi employ secondary metabolites (SMs) to cope with the external biotic environment. Successive steps of SMs biosynthetic pathways are likely to be clustered in the genomes of bacteria, fungi, and even plants (Osbourn, 2010). In our analysis, *Protomyces* spp. genomes displayed multiple predicted secondary metabolite gene clusters. The assumed products are terpenes and non-ribosomal peptides (NRPs). Each *Protomyces* species has one NRP and four terpenes gene clusters, except for Plac which has only three terpene clusters. Gene synteny of seven *Protomyces* spp. NRP gene clusters are shown in **Fig. 2**. Highly conserved gene synteny exists between Pinu and Pmac, as well as among SC29, Pino, Plac, and Ppac. Pgra NRPS gene clusters appears to be dissimilar to other *Protomyces* spp. The core gene of each NRP gene cluster is NRP synthetase (g5034 in Pinu), which is sitting next to Flap endonuclease 1 (g5033 in Pinu), MFS transporter (g5035 in Pinu) and other related proteins. Gene synteny of four terpenes gene clusters were also analyzed. Terpenes gene synteny similarity (not shown) among *Protomyces* spp. behaves similar to NRPs, indicating that in *Protomyces* spp. multiple SM gene clusters likely evolve in a conserved way, though Pgra exhibits differences.

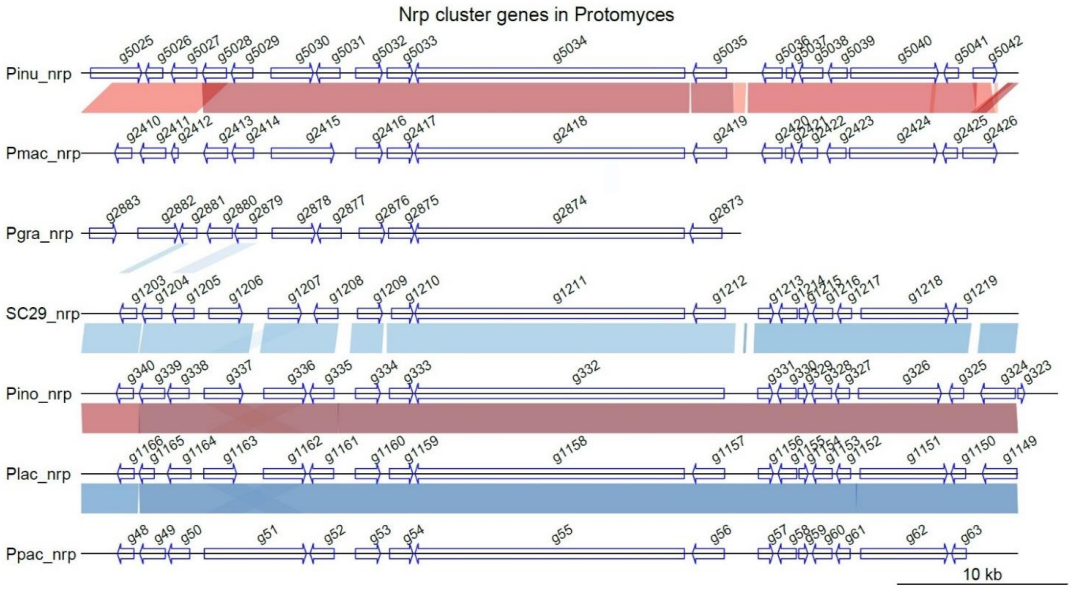

Figure 2. Gene synteny of *Protomyces* NRPS (non-ribosomal peptide synthase) gene clusters. Cluster genes were predicted with online tool antiSMASH (fungal). Plots were viewed with package genoPlotR in R (3.5.1). Comparison was performed with BLASTn among DNA sequences of contigs. Red color indicates the same sequence order, blue color indicates reverse sequence order. Color strength indicates level of DNA homology.

4.4 SC29 as a novel *Protomyces* species

As mentioned above in section 1.4.1, this research included all the available specimen of *Protomyces* species, although many more species have been proposed (Reddy and Kramer, 1975, Kurtzman, 2011). In the genome-wide phylogeny tree built with 1670 single-copy protein sequences, SC29 holds a separate unique position among the *Protomyces* (**III**, Figure 2, a). The phylogenetic distance between SC29 and *P. inouyei* is larger than those between *P. inouyei*, *P. lactucaedebilis* and *P. pachydermus* (**III**, Figure 2, a). As expected, the high similarity of ITS or D1/D2 sequences do not reflect the true phylogenetic diversity among *Protomyces* spp. (**III**, Figure 2, b and c). Phylogenetic trees built with nuclear gene DNA markers, such as *ACT1*, *TUB2*, *RPB1*, *RPB2*, and *TEF1*, also indicated the divergent placement of SC29 to other tested *Protomyces* spp. (**III,** Figure S2). Among those markers, *ACT1* exhibited the identical phylogenetic architecture as seen in the genome-wide tree. Thus, *ACT1* seems the best single gene marker that clearly reflects the phylogenetic relation among genus *Protomyces*. By virtue of the unique position of SC29 in multiple phylogeny tree and low ANI and AAI values against other six described *Protomyces* spp. (III, Figure 2; Table 1; Figure S2), SC29 appears to be genomically distinct from other *Protomyces* spp. Cell and colony morphology of SC29 are also different from the most closely related species (**II**, Figure S1; Table S1). Additionally, all tested *Protomyces* spp., including SC29, have differential carbon assimilation patterns (**II**, Table S2). Taken together, we proposed a new species name *P. arabidopsicola* with SC29 as the type strain.

P. inouyei and *P. lactucaedebilis* have over 96% ANI and 97% AAI (**III**, Table 1), which exceed the boundary, under which they could be considered different species. Moreover, an extremely high level of genome synteny between *P. inouyei* and *P. lactucaedebilis* was seen with our genome assembly data (**III**, Figure S1). Given the fact that *P. inouyei* and *P. lactucaedebilis* have high genome similarity, we propose these two species are merged into one species. Thus, we propose the species name *P. lactucaedebilis* should be renamed as *P. inouyei* f. sp. *lactucae.* In addition, *P. inouyei* should also be renamed to *P. inouyei f. sp. crepis*.

5 Conclusion and future perspectives

Plant associated yeast stains were isolated from the leaf surface of wild growing *Arabidopsis*, offering about a hundred isolates, which are excellent resources for the study of plant-yeast interaction with the genetic model plant *Arabidopsis*. Interestingly, *Protomyces* and *Taphrina* species are present. Several important physiological aspects have been characterized. Camalexin sensitivity test suggests that most phyllosphere yeast residents are highly tolerant to camalexin. Indolic compounds are produced by many of the yeast strains in our collection.

With deep investigation of the genus *Protomyces*, we conclude that *Protomyces* sp. SC29 (SC29) represents a novel species. SC29 has a unique evolutionary position and distinct genome similarity among other *Protomyces* species. The carbon assimilation pattern of SC29 also support its uniqueness. Most strikingly, SC29 persists on *Arabidopsis* leaf surface and activates plant immunity, while cell counts of its closest relative *P. inouyei* drops down rapidly. The indolic compounds production of *Protomyces* species are all significantly boosted when provided with additional tryptophan, indicating the importance of tryptophan-dependent auxin synthesis pathway. Moreover, the indole-3-pyruvic acid (IPyA) auxin synthesis pathway is present in all *Protomyces* species with genes encoding key enzymes were found. The genomic assemblies and characters of seven *Protomyces* species were present in this work. SC29-specific and *Protomyces*-specific characters have been discovered. SC29 has phyllosphere adaptation signatures in its genomic content, including gene ontology enrichment in DNA repair and gene loss in invasive filamentous growth. The expansion of group I sensory histidine kinase in SC29 might be related to the lifestyle change. Thus, we propose SC29 as a new *Protomyces* species *Protomyces arabidopsicola*.

The presence of SC29 on *Arabidopsis* leaf surface activates plant immunity, with upregulated SA signaling and camalexin biosynthesis pathways, as well as activated MAPK3/6 involvement. The future question is to understand the mechanism of how SC29 survive on leaf surface and how it interacts with *Arabidopsis* immunity. Lacking LysM effector suggests that there is little possibility that chitin is the major *Protomyces* PAMP recognized by *Arabidopsis*. On the other hand, mannose or other cell wall components could be significant yeast PAMPs. This study has built a yeast-*Arabidopsis* interaction system that allows the use of *Arabidopsis* as a model genetic plant to study the genetics of plant yeast interactions. Uncovering of the PAMP from SC29 and corresponding pattern recognition receptors in *Arabidopsis* in the future would be exciting significant advance, since the yeast PAMPs and related plant PRRs are completely unknown. Additionally, the roles of phytohormone auxin in plant-yeast interaction is another area with rich opportunities to be explored.

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Reference

Abrudan, M. I., Smakman, F., Grimbergen, A. J., Westhoff, S., Miller, E. L., Van Wezel, G. P. & Rozen, D. E. 2015. Socially mediated induction and suppression of antibiosis during bacterial coexistence. *Proceedings of the National Academy of Sciences,* 112**,** 11054-11059.

Achard, P., Vriezen, W. H., Van Der Straeten, D. & Harberd, N. P. 2003. Ethylene regulates Arabidopsis development via the modulation of DELLA protein growth repressor function. *The Plant Cell,* 15**,** 2816-2825.

Ádám, A., Nagy, Z., Kátay, G., Mergenthaler, E. & Viczián, O. 2018. Signals of systemic immunity in plants: Progress and open questions. *International journal of molecular sciences,* 19**,** 1146.

Agler, M. T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D. & Kemen, E. M. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol,* 14**,** e1002352.

Ahlfors, R., Macioszek, V., Rudd, J., Brosché, M., Schlichting, R., Scheel, D. & Kangasjärvi, J. 2004. Stress hormone‐independent activation and nuclear translocation of mitogen‐activated protein kinases in *Arabidopsis thaliana* during ozone exposure. *The Plant Journal,* 40**,** 512-522.

Aragón, W., Reina-Pinto, J. J. & Serrano, M. 2017. The intimate talk between plants and microorganisms at the leaf surface. *Journal of experimental botany,* 68**,** 5339-5350.

Arbibe, L., Kim, D. W., Batsche, E., Pedron, T., Mateescu, B., Muchardt, C., Parsot, C. & Sansonetti, P. J. 2007. An injected bacterial effector targets chromatin access for transcription factor NF-κB to alter transcription of host genes involved in immune responses. *Nature immunology,* 8**,** 47.

Asai, S., Ohta, K. & Yoshioka, H. 2008. MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. *The Plant Cell,* 20**,** 1390-1406.

Asai, S. & Yoshioka, H. 2008. The role of radical burst via MAPK signaling in plant immunity. *Plant Signaling & Behavior,* 3**,** 920-922.

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W.-L., Gomez-Gomez, L., Boller, T., Ausubel, F. M. & Sheen, J. 2002. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature,* 415**,** 977.

Ausubel, F. M. 2005. Are innate immune signaling pathways in plants and animals conserved? *Nature immunology,* 6**,** 973.

Bacigálová, K. 2008. *Protomyces buerenianus* (Protomycetaceae)—a new species for Slovakia. *Biologia,* 63**,** 40-43.

Bacigálová, K., Piątek, M. & Wołkowycki, M. 2005. *Protomyces cirsii-oleracei* (Fungi, Protomycetales), a new species for Poland. *Polish Botanical Journal,* 50**,** 77-82.

Bajguz, A. & Hayat, S. 2009. Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry,* 47**,** 1-8.

Baltrus, D. A., Nishimura, M. T., Romanchuk, A., Chang, J. H., Mukhtar, M. S., Cherkis, K., Roach, J., Grant, S. R., Jones, C. D. & Dangl, J. L. 2011. Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. *PLoS pathogens,* 7**,** e1002132.

Beckers, G. J., Jaskiewicz, M., Liu, Y., Underwood, W. R., He, S. Y., Zhang, S. & Conrath, U. 2009. Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *The Plant Cell,* 21**,** 944-953.

Begerow, D., Nilsson, H., Unterseher, M. & Maier, W. 2010. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology,* 87**,** 99-108.

Beneloujaephajri, E., Costa, A., L'haridon, F., Métraux, J.-P. & Binda, M. 2013. Production of reactive oxygen species and wound-induced resistance in *Arabidopsis thaliana* against *Botrytis cinerea* are preceded and depend on a burst of calcium. *BMC plant biology,* 13**,** 160.

Birkenbihl, R. P., Kracher, B., Roccaro, M. & Somssich, I. E. 2017. Induced genome-wide binding of three Arabidopsis WRKY transcription factors during early MAMP-triggered immunity. *The Plant Cell,* 29**,** 20-38.

Birkenbihl, R. P., Kracher, B., Ross, A., Kramer, K., Finkemeier, I. & Somssich, I. E. 2018. Principles and characteristics of the Arabidopsis WRKY regulatory network during early MAMP‐triggered immunity. *The Plant Journal,* 96**,** 487-502.

Blackwell, M. 2011. The Fungi: 1, 2, 3… 5.1 million species? *American journal of botany,* 98**,** 426-438.
Bogdanove, A. J., Schornack, S. & Lahaye, T. 2010. TAL effectors: finding plant genes for disease and defense. *Current opinion in plant biology,* 13**,** 394-401.
Bradley, D. J., Kjellbom, P. & Lamb, C. J. 1992. Elicitor-and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. *Cell,* 70**,** 21-30.
Brandl, M. & Lindow, S. 1998. Contribution of indole-3-acetic acid production to the epiphytic fitness of *Erwinia herbicola*. *Appl. Environ. Microbiol.,* 64**,** 3256-3263.
Brodersen, P., Petersen, M., Nielsen, H. B., Zhu, S., Newman, M. A., Shokat, K. M., Rietz, S., Parker, J. & Mundy, J. 2006. Arabidopsis MAP kinase 4 regulates salicylic acid - and jasmonic acid/ethylene - dependent responses via EDS1 and PAD4. *The Plant Journal,* 47**,** 532-546.
Brutus, A., Sicilia, F., Macone, A., Cervone, F. & De Lorenzo, G. 2010. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proceedings of the National Academy of Sciences,* 107**,** 9452-9457.
Buist, G., Steen, A., Kok, J. & Kuipers, O. P. 2008. LysM, a widely distributed protein motif for binding to (peptido) glycans. *Molecular microbiology,* 68**,** 838-847.
Burch, A. Y., Zeisler, V., Yokota, K., Schreiber, L. & Lindow, S. E. 2014. The hygroscopic biosurfactant syringafactin produced by *Pseudomonas syringae* enhances fitness on leaf surfaces during fluctuating humidity. *Environmental microbiology,* 16**,** 2086-2098.
Buxdorf, K., Rahat, I., Gafni, A. & Levy, M. 2013. The epiphytic fungus *Pseudozyma aphidis* induces jasmonic acid- and salicylic acid/Nonexpressor of PR1-independent local and systemic resistance. *Plant Physiol,* 161**,** 2014-2022.
Cao, Y., Liang, Y., Tanaka, K., Nguyen, C. T., Jedrzejczak, R. P., Joachimiak, A. & Stacey, G. 2014. The kinase LYK5 is a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase CERK1. *Elife,* 3**,** e03766.
Cartwright, R. & Templeton, G. 1988. Biological limitations of *Protomyces gravidus* as a mycoherbicide for giant ragweed, *Ambrosia trifida*. *Plant Disease,* 72**,** 580-582.
Century, K. S., Holub, E. B. & Staskawicz, B. J. 1995. *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. *Proceedings of the National Academy of Sciences,* 92**,** 6597-6601.
Chassot, C., Nawrath, C. & Métraux, J. P. 2007. Cuticular defects lead to full immunity to a major plant pathogen. *The Plant Journal,* 49**,** 972-980.
Chen, Z., Kloek, A. P., Cuzick, A., Moeder, W., Tang, D., Innes, R. W., Klessig, D. F., Mcdowell, J. M. & Kunkel, B. N. 2004. The *Pseudomonas syringae* type III effector AvrRpt2 functions downstream or independently of SA to promote virulence on *Arabidopsis thaliana*. *The Plant Journal,* 37**,** 494-504.
Chiang, Y.-H. & Coaker, G. 2015. Effector triggered immunity: NLR immune perception and downstream defense responses. *The Arabidopsis Book,* 2015.
Chinchilla, D., Shan, L., He, P., De Vries, S. & Kemmerling, B. 2009. One for all: the receptor-associated kinase BAK1. *Trends in plant science,* 14**,** 535-541.
Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nürnberger, T., Jones, J. D. G., Felix, G. & Boller, T. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature,* 448**,** 497.
Chisholm, S. T., Coaker, G., Day, B. & Staskawicz, B. J. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell,* 124**,** 803-814.
Choi, J., Huh, S. U., Kojima, M., Sakakibara, H., Paek, K.-H. & Hwang, I. 2010. The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in *Arabidopsis*. *Developmental cell,* 19**,** 284-295.
Choi, J., Tanaka, K., Liang, Y., Cao, Y., Lee, S. Y. & Stacey, G. 2014. Extracellular ATP, a danger signal, is recognized by DORN1 in *Arabidopsis*. *Biochemical Journal,* 463**,** 429-437.
Choudhary, S. P., Yu, J.-Q., Yamaguchi-Shinozaki, K., Shinozaki, K. & Tran, L.-S. P. 2012. Benefits of brassinosteroid crosstalk. *Trends in plant science,* 17**,** 594-605.
Cimmino, A., Andolfi, A. & Evidente, A. 2014. Phytotoxic terpenes produced by phytopathogenic fungi and allelopathic plants. *Natural product communications,* 9**,** 1934578X1400900330.

Cissé, O. H., Almeida, J. M., Fonseca, Á., Kumar, A. A., Salojärvi, J., Overmyer, K., Hauser, P. M. & Pagni, M. 2013. Genome sequencing of the plant pathogen *Taphrina deformans*, the causal agent of peach leaf curl. *MBio,* 4**,** e00055-13.

Colcombet, J. & Hirt, H. 2008. *Arabidopsis* MAPKs: a complex signalling network involved in multiple biological processes. *Biochemical Journal,* 413**,** 217-226.

Cui, H., Gobbato, E., Kracher, B., Qiu, J., Bautor, J. & Parker, J. E. 2017. A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in Arabidopsis immunity. *New Phytologist,* 213**,** 1802-1817.

Cui, H., Tsuda, K. & Parker, J. E. 2015. Effector-triggered immunity: from pathogen perception to robust defense. *Annual review of plant biology,* 66**,** 487-511.

Dalmais, B., Schumacher, J., Moraga, J., Le Pecheur, P., Tudzynski, B., Collado, I. G. & Viaud, M. 2011. The *Botrytis cinerea* phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial. *Molecular plant pathology,* 12**,** 564-579.

Dangl, J. L. & Jones, J. D. 2019. A pentangular plant inflammasome. *Science,* 364**,** 31-32.

Davis, J. 1907. A new species of *Protomyces*. *The Journal of Mycology,* 13**,** 188-189.

De Azeredo, L. a. I., Gomes, E. a. T., Mendonça-Hagler, L. C. & Hagler, A. N. 2010. Yeast communities associated with sugarcane in Campos, Rio de Janeiro, Brazil. *International Microbiology,* 1**,** 205-208.

De Jonge, R., Van Esse, H. P., Kombrink, A., Shinya, T., Desaki, Y., Bours, R., Van Der Krol, S., Shibuya, N., Joosten, M. H. & Thomma, B. P. 2010. Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. *Science,* 329**,** 953-955.

De Torres‐Zabala, M., Truman, W., Bennett, M. H., Lafforgue, G., Mansfield, J. W., Egea, P. R., Bögre, L. & Grant, M. 2007. *Pseudomonas syringae* pv. tomato hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *The EMBO journal,* 26**,** 1434-1443.

Decreux, A. & Messiaen, J. 2005. Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. *Plant Cell Physiology,* 46**,** 268-278.

Defosse, T. A., Sharma, A., Mondal, A. K., Dugé De Bernonville, T., Latgé, J. P., Calderone, R., Giglioli‐Guivarc'h, N., Courdavault, V., Clastre, M. & Papon, N. 2015. Hybrid histidine kinases in pathogenic fungi. *Molecular microbiology,* 95**,** 914-924.

Delaye, L., García-Guzmán, G. & Heil, M. 2013. Endophytes versus biotrophic and necrotrophic pathogens-are fungal lifestyles evolutionarily stable traits? *Fungal Diversity,* 60**,** 125-135.

Demidchik, V. & Shabala, S. 2018. Mechanisms of cytosolic calcium elevation in plants: the role of ion channels, calcium extrusion systems and NADPH oxidase-mediated 'ROS-Ca^{2+} Hub'. *Functional Plant Biology,* 45**,** 9-27.

Demidchik, V., Shabala, S., Isayenkov, S., Cuin, T. A. & Pottosin, I. 2018. Calcium transport across plant membranes: mechanisms and functions. *New Phytologist,* 220**,** 49-69.

Denoux, C., Galletti, R., Mammarella, N., Gopalan, S., Werck, D., De Lorenzo, G., Ferrari, S., Ausubel, F. M. & Dewdney, J. 2008. Activation of defense response pathways by OGs and Flg22 elicitors in Arabidopsis seedlings. *Molecular Plant,* 1**,** 423-445.

Depoorter, E., Bull, M. J., Peeters, C., Coenye, T., Vandamme, P. & Mahenthiralingam, E. 2016. *Burkholderia*: an update on taxonomy and biotechnological potential as antibiotic producers. *Applied microbiology and biotechnology,* 100**,** 5215-5229.

Desaki, Y., Kohari, M., Shibuya, N. & Kaku, H. 2019. MAMP-triggered plant immunity mediated by the LysM-receptor kinase CERK1. *Journal of General Plant Pathology***,** 1-11.

Ding, Y., Dommel, M. & Mou, Z. 2016. Abscisic acid promotes proteasome‐mediated degradation of the transcription coactivator NPR 1 in *Arabidopsis thaliana*. *The Plant Journal,* 86**,** 20-34.

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y., Li, X. & Zhang, Y. 2018. Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. *Cell,* 173**,** 1454-1467. e15.

Dou, D., Kale, S. D., Wang, X., Jiang, R. H., Bruce, N. A., Arredondo, F. D., Zhang, X. & Tyler, B. M. 2008. RXLR-mediated entry of *Phytophthora sojae* effector *Avr1b* into soybean cells does not require pathogen-encoded machinery. *The Plant Cell,* 20**,** 1930-1947.

Droillard, M.-J., Boudsocq, M., Barbier-Brygoo, H. & Laurière, C. 2002. Different protein kinase families are activated by osmotic stresses in *Arabidopsis thaliana* cell suspensions. *FEBS letters,* 527**,** 43-50.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C. & Lepiniec, L. 2010. MYB transcription factors in *Arabidopsis*. *Trends in plant science,* 15**,** 573-581.

Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P. & Hacquard, S. 2018. Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell,* 175**,** 973-983. e14.

Esmaeel, Q., Pupin, M., Jacques, P. & Leclère, V. 2018. Nonribosomal peptides and polyketides of *Burkholderia*: New compounds potentially implicated in biocontrol and pharmaceuticals. *Environmental Science Pollution Research,* 25**,** 29794-29807.

Felix, G., Duran, J. D., Volko, S. & Boller, T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *The Plant Journal,* 18**,** 265-276.

Fell, J. W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *International journal of systematic and evolutionary microbiology,* 50**,** 1351-1371.

Feng, F., Yang, F., Rong, W., Wu, X., Zhang, J., Chen, S., He, C. & Zhou, J.-M. 2012. A *Xanthomonas* uridine 5′-monophosphate transferase inhibits plant immune kinases. *Nature,* 485**,** 114.

Finn, R. D., Coggill, P., Eberhardt, R. Y., Eddy, S. R., Mistry, J., Mitchell, A. L., Potter, S. C., Punta, M., Qureshi, M. & Sangrador-Vegas, A. 2015. The Pfam protein families database: towards a more sustainable future. *Nucleic acids research,* 44**,** D279-D285.

Fleischmann, R. D., Adams, M. D., White, O., Clayton, R. A., Kirkness, E. F., Kerlavage, A. R., Bult, C. J., Tomb, J.-F., Dougherty, B. A. & Merrick, J. M. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science,* 269**,** 496-512.

Fleming, A. 1929. On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzæ*. *British journal of experimental pathology,* 10**,** 226-236.

Fonseca, Á. & Rodrigues, M. G. 2011. *Taphrina* Fries (1832). *In:* KURTZMAN, C. P., FELL, J. W. & BOEKHOUT, T. (eds.) *The yeasts: a taxonomic study.* 5th ed.: Elsevier.

Fu, Z. Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S. H., Tada, Y., Zheng, N. & Dong, X. 2012. NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature,* 486**,** 228.

Gimenez-Ibanez, S., Hann, D. R., Ntoukakis, V., Petutschnig, E., Lipka, V. & Rathjen, J. P. 2009. AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. *Current biology,* 19**,** 423-429.

Godfrey, D., Böhlenius, H., Pedersen, C., Zhang, Z., Emmersen, J. & Thordal-Christensen, H. 2010. Powdery mildew fungal effector candidates share N-terminal Y/F/WxC-motif. *Bmc Genomics,* 11**,** 317.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J., Jacq, C. & Johnston, M. 1996. Life with 6000 genes. *Science,* 274**,** 546-567.

Golubev, W., Kulakovskaya, T., Shashkov, A., Kulakovskaya, E. & Golubev, N. 2008. Antifungal cellobiose lipid secreted by the epiphytic yeast *Pseudozyma graminicola*. *Microbiology,* 77**,** 171-175.

Gourion, B., Rossignol, M. & Vorholt, J. A. 2006. A proteomic study of *Methylobacterium extorquens* reveals a response regulator essential for epiphytic growth. *Proceedings of the National Academy of Sciences,* 103**,** 13186-13191.

Grant, M., Brown, I., Adams, S., Knight, M., Ainslie, A. & Mansfield, J. 2000. The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. *The Plant Journal,* 23**,** 441-450.

Gu, Y., Zebell, S. G., Liang, Z., Wang, S., Kang, B.-H. & Dong, X. 2016. Nuclear pore permeabilization is a convergent signaling event in effector-triggered immunity. *Cell,* 166**,** 1526-1538. e11.

Gudesblat, G. E., Iusem, N. D. & Morris, P. C. 2007. Guard cell‐specific inhibition of *Arabidopsis MPK3* expression causes abnormal stomatal responses to abscisic acid and hydrogen peroxide. *New Phytologist,* 173**,** 713-721.

Gunasekera, T. & Sundin, G. 2006. Role of nucleotide excision repair and photoreactivation in the solar UVB radiation survival of *Pseudomonas syringae* pv. *syringae* B728a. *Journal of applied microbiology,* 100**,** 1073-1083.

Haas, H., Eisendle, M. & Turgeon, B. G. 2008. Siderophores in fungal physiology and virulence. *Annu. Rev. Phytopathol.,* 46**,** 149-187.

Hamel, L.-P., Nicole, M.-C., Sritubtim, S., Morency, M.-J., Ellis, M., Ehlting, J., Beaudoin, N., Barbazuk, B., Klessig, D. & Lee, J. 2006. Ancient signals: comparative genomics of plant MAPK and MAPKK gene families. *Trends in plant science,* 11**,** 192-198.

Hartmann, M., Zeier, T., Bernsdorff, F., Reichel-Deland, V., Kim, D., Hohmann, M., Scholten, N., Schuck, S., Bräutigam, A. & Hölzel, T. 2018. Flavin monooxygenase-generated N-hydroxypipecolic acid is a critical element of plant systemic immunity. *Cell,* 173**,** 456-469. e16.

Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Eugene, C. Y., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M. & Aderem, A. 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature,* 410**,** 1099.

He, P., Shan, L., Lin, N.-C., Martin, G. B., Kemmerling, B., Nürnberger, T. & Sheen, J. 2006. Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in *Arabidopsis* innate immunity. *Cell,* 125**,** 563-575.

Heese, A., Hann, D. R., Gimenez-Ibanez, S., Jones, A. M., He, K., Li, J., Schroeder, J. I., Peck, S. C. & Rathjen, J. P. 2007. The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences,* 104**,** 12217-12222.

Helaly, S. E., Thongbai, B. & Stadler, M. 2018. Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. *Natural product reports,* 35**,** 992-1014.

Helfrich, E. J., Vogel, C. M., Ueoka, R., Schäfer, M., Ryffel, F., Müller, D. B., Probst, S., Kreuzer, M., Piel, J. & Vorholt, J. A. 2018. Bipartite interactions, antibiotic production and biosynthetic potential of the *Arabidopsis* leaf microbiome. *Nature microbiology,* 3**,** 909.

Holcomb, G. 1995. First report of *Protomyces gravidus* causing stem galls on giant ragweed in Louisiana. *Plant Disease,* 79.

Howard, R. J. & Valent, B. 1996. Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. *Annual Reviews in Microbiology,* 50**,** 491-512.

Irieda, H., Inoue, Y., Mori, M., Yamada, K., Oshikawa, Y., Saitoh, H., Uemura, A., Terauchi, R., Kitakura, S. & Kosaka, A. 2019. Conserved fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. *Proceedings of the National Academy of Sciences,* 116**,** 496-505.

Jacob, S., Foster, A. J., Yemelin, A. & Thines, E. 2014. Histidine kinases mediate differentiation, stress response, and pathogenicity in *Magnaporthe oryzae*. *Microbiologyopen,* 3**,** 668-687.

Jacobs, J., Carroll, T. & Sundin, G. 2005. The role of pigmentation, ultraviolet radiation tolerance, and leaf colonization strategies in the epiphytic survival of phyllosphere bacteria. *Microbial Ecology,* 49**,** 104-113.

Jalmi, S. K. & Sinha, A. K. 2015. ROS mediated MAPK signaling in abiotic and biotic stress-striking similarities and differences. *Frontiers in plant science,* 6**,** 769.

Jameson, P. E. 2000. Cytokinins and auxins in plant-pathogen interactions – An overview. *Plant Growth Regulation,* 32**,** 369-380.

Jeter, C. R., Tang, W., Henaff, E., Butterfield, T. & Roux, S. J. 2004. Evidence of a novel cell signaling role for extracellular adenosine triphosphates and diphosphates in Arabidopsis. *The Plant Cell,* 16**,** 2652-2664.

Jia, L.-J., Tang, H.-Y., Wang, W.-Q., Yuan, T.-L., Wei, W.-Q., Pang, B., Gong, X.-M., Wang, S.-F., Li, Y.-J., Zhang, D., Liu, W. & Tang, W.-H. 2019. A linear nonribosomal octapeptide from Fusarium graminearum facilitates cell-to-cell invasion of wheat. *Nature Communications,* 10**,** 922.

Jiang, J., Ma, S., Ye, N., Jiang, M., Cao, J. & Zhang, J. 2017. WRKY transcription factors in plant responses to stresses. *Journal of integrative plant biology,* 59**,** 86-101.

Jones, D. A., Bertazzoni, S., Turo, C. J., Syme, R. A. & Hane, J. K. 2018. Bioinformatic prediction of plant–pathogenicity effector proteins of fungi. *Current opinion in microbiology,* 46**,** 43-49.

Jones, J. D. & Dangl, J. L. 2006. The plant immune system. *Nature,* 444**,** 323-329.

Jumpponen, A. & Jones, K. 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytologist,* 184**,** 438-448.

Junker, C., Draeger, S. & Schulz, B. 2012. A fine line-endophytes or pathogens in *Arabidopsis thaliana*. *Fungal Ecology,* 5**,** 657-662.

Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V., Jones, J. D., Shirasu, K., Menke, F. & Jones, A. 2014. Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. *Molecular cell,* 54**,** 43-55.

Kale, S. D., Gu, B., Capelluto, D. G., Dou, D., Feldman, E., Rumore, A., Arredondo, F. D., Hanlon, R., Fudal, I. & Rouxel, T. 2010. External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. *Cell,* 142**,** 284-295.

Kay, S., Hahn, S., Marois, E., Hause, G. & Bonas, U. 2007. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science,* 318**,** 648-651.

Kazan, K. & Lyons, R. 2014. Intervention of phytohormone pathways by pathogen effectors. *The Plant Cell,* 26**,** 2285-2309.

Kemen, A. C., Agler, M. T. & Kemen, E. 2015. Host–microbe and microbe–microbe interactions in the evolution of obligate plant parasitism. *New Phytologist,* 206**,** 1207-1228.

Khaldi, N., Seifuddin, F. T., Turner, G., Haft, D., Nierman, W. C., Wolfe, K. H. & Fedorova, N. D. 2010. SMURF: genomic mapping of fungal secondary metabolite clusters. *Fungal Genetics and Biology,* 47**,** 736-741.

Khan, M. R. & Parveen, G. 2018. Supplementing biocontrol agents with botanicals improved growth and yield of coriander (*Coriandrum sativum* L.) infected with *Protomyces macrosporus* Unger. *Current Plant Biology,* 15**,** 44-50.

Kjærbølling, I., Vesth, T. C., Frisvad, J. C., Nybo, J. L., Theobald, S., Kuo, A., Bowyer, P., Matsuda, Y., Mondo, S. & Lyhne, E. K. 2018. Linking secondary metabolites to gene clusters through genome sequencing of six diverse *Aspergillus* species. *Proceedings of the National Academy of Sciences,* 115**,** E753-E761.

Klessig, D. F. 2012. SOS-too many signals for systemic acquired resistance? *Trends in plant science,* 17**,** 538-545.

Kobayashi, M., Ohura, I., Kawakita, K., Yokota, N., Fujiwara, M., Shimamoto, K., Doke, N. & Yoshioka, H. 2007. Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. *The plant cell,* 19**,** 1065-1080.

Kombrink, A. & Thomma, B. P. 2013. LysM effectors: secreted proteins supporting fungal life. *PLoS pathogens,* 9**,** e1003769.

Kubicek, C. P., Starr, T. L. & Glass, N. L. 2014. Plant cell wall–degrading enzymes and their secretion in plant-pathogenic fungi. *Annual review of phytopathology,* 52**,** 427-451.

Kurtzman, C. P. 2011. *Protomyces* Unger (1833). *In:* KURTZMAN, C. P., FELL, J. W. & BOEKHOUT, T. (eds.) *The yeasts: a taxonomic study.* 5th ed.: Elsevier.

Kurtzman, C. P., Fell, J. W. & Boekhout, T. 2011. *The yeasts: a taxonomic study*, Elsevier.

Kutschera, A., Dawid, C., Gisch, N., Schmid, C., Raasch, L., Gerster, T., Schäffer, M., Smakowska-Luzan, E., Belkhadir, Y., Vlot, A. C., Chandler, C. E., Schellenberger, R., Schwudke, D., Ernst, R. K., Dorey, S., Hückelhoven, R., Hofmann, T. & Ranf, S. 2019. Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in *Arabidopsis* plants. *Science,* 364**,** 178-181.

Kämper, J., Kahmann, R., Bölker, M., Ma, L.-J., Brefort, T., Saville, B. J., Banuett, F., Kronstad, J. W., Gold, S. E. & Müller, O. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature,* 444**,** 97-101.

Last, F. 1955. Seasonal incidence of *Sporobolomyces* on cereal leaves. *Transactions of the British Mycological Society,* 38**,** 221-239.

Last, F. & Deighton, F. 1965. The non-parasitic microflora on the surfaces of living leaves. *Transactions of the British Mycological Society,* 48**,** 83-IN12.

Leben, C. 1965. Epiphytic microorganisms in relation to plant disease. *Annual Review of phytopathology,* 3**,** 209-230.

Lee, G., Lee, S.-H., Kim, K. M. & Ryu, C.-M. 2017. Foliar application of the leaf-colonizing yeast *Pseudozyma churashimaensis* elicits systemic defense of pepper against bacterial and viral pathogens. *Scientific reports,* 7**,** 39432.

Leveau, J. H. & Lindow, S. E. 2001. Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences,* 98**,** 3446-3453.

Li, G., Meng, X., Wang, R., Mao, G., Han, L., Liu, Y. & Zhang, S. 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in Arabidopsis. *PLoS genetics,* 8**,** e1002767.

Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., Cai, G., Gao, L., Zhang, X. & Wang, Y. 2014. The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell host & microbe,* 15**,** 329-338.

Li, Z., De La Torre, A. R., Sterck, L., Cánovas, F. M., Avila, C., Merino, I., Cabezas, J. A., Cervera, M. T., Ingvarsson, P. K. & Van De Peer, Y. 2017. Single-copy genes as molecular markers for phylogenomic studies in seed plants. *Genome Biology and Evolution,* 9**,** 1130-1147.

Limtong, S. & Kaewwichian, R. 2015. The diversity of culturable yeasts in the phylloplane of rice in Thailand. *Annals of Microbiology,* 65**,** 667-675.

Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R. P. & Somssich, I. E. 2015. Negative regulation of ABA signaling by WRKY33 is critical for *Arabidopsis* immunity towards *Botrytis cinerea* 2100. *Elife,* 4**,** e07295.

Liu, T., Song, T., Zhang, X., Yuan, H., Su, L., Li, W., Xu, J., Liu, S., Chen, L. & Chen, T. 2014. Unconventionally secreted effectors of two filamentous pathogens target plant salicylate biosynthesis. *Nature communications,* 5**,** 4686.

Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L. & He, P. 2010. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proceedings of the National Academy of Sciences,* 107**,** 496-501.

Ma, Z., Zhu, L., Song, T., Wang, Y., Zhang, Q., Xia, Y., Qiu, M., Lin, Y., Li, H. & Kong, L. 2017. A paralogous decoy protects *Phytophthora sojae* apoplastic effector PsXEG1 from a host inhibitor. *Science,* 355**,** 710-714.

Malhotra, S., Kakani, R., Sharma, Y., Saxena, S. & Vashishtha, B. 2016. Comparative superiority of coriander variety NRCSS ACr-1 for yield and stem gall disease tolerance. *Indian Journal of Horticulture,* 73**,** 453-455.

Mccormack, P. J., Wildman, H. G. & Jeffries, P. 1994. Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeastlike fungi. *Appl. Environ. Microbiol.,* 60**,** 927-931.

Melin, P., Schnürer, J. & Wagner, E. 2002. Proteome analysis of *Aspergillus nidulans* reveals proteins associated with the response to the antibiotic concanamycin A, produced by *Streptomyces* species. *Molecular Genetics and Genomics,* 267**,** 695-702.

Meng, X. & Zhang, S. 2013. MAPK cascades in plant disease resistance signaling. *Annual review of phytopathology,* 51**,** 245-266.

Meyers, B. C., Kozik, A., Griego, A., Kuang, H. & Michelmore, R. W. 2003. Genome-wide analysis of NBS-LRR–encoding genes in Arabidopsis. *The Plant Cell,* 15**,** 809-834.

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H. & Shibuya, N. 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proceedings of the National Academy of Sciences,* 104**,** 19613-19618.

Moon, H., Lee, G., Yun, H. S. & Kwon, C. 2015. Non-proteinaceous yeast extract induces arabidopsis defense responses independently of salicylic acid. *Journal of Plant Biology,* 58**,** 38-43.

Mukhtar, M. S., Carvunis, A.-R., Dreze, M., Epple, P., Steinbrenner, J., Moore, J., Tasan, M., Galli, M., Hao, T. & Nishimura, M. T. 2011. Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science,* 333**,** 596-601.

Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., Sekimata, K., Takatsuto, S., Yamaguchi, I. & Yoshida, S. 2003. Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *The Plant Journal,* 33**,** 887-898.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. & Jones, J. D. 2006. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science,* 312**,** 436-439.

Navarro, L., Jay, F., Nomura, K., He, S. Y. & Voinnet, O. 2008. Suppression of the microRNA pathway by bacterial effector proteins. *Science,* 321**,** 964-967.

Netzker, T., Fischer, J., Weber, J., Mattern, D. J., Koenig, C. C., Valiante, V., Schroeckh, V. & Brakhage, A. A. 2015. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. *Frontiers in microbiology,* 6**,** 299.

Nilsson, R. H., Hyde, K. D., Pawłowska, J., Ryberg, M., Tedersoo, L., Aas, A. B., Alias, S. A., Alves, A., Anderson, C. L. & Antonelli, A. 2014. Improving ITS sequence data for identification of plant pathogenic fungi. *Fungal Diversity,* 67**,** 11-19.

Nishida, H., Blanz, P. A. & Sugiyama, J. 1993. The higher fungus *Protomyces inouyei* has two group I introns in the 18S rRNA gene. *Journal of molecular evolution,* 37**,** 25-28.

Nishida, H. & Sugiyama, J. 1995. A common group I intron between a plant parasitic fungus and its host. *Molecular Biology and Evolution,* 12**,** 883-886.

Nishida, H., Tajiri, Y. & Sugiyama, J. 1998. Multiple origins of fungal group I introns located in the same position of nuclear SSU rRNA gene. *Journal of molecular evolution,* 46**,** 442-448.

Noctor, G., Reichheld, J.-P. & Foyer, C. H. ROS-related redox regulation and signaling in plants. Seminars in cell & developmental biology, 2017. Elsevier, 3-12.

Onofre-Lemus, J., Hernández-Lucas, I., Girard, L. & Caballero-Mellado, J. 2009. ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth-promoting effect on tomato plants. *Applied and Environment Microbiology,* 75**,** 6581-6590.

Osbourn, A. 2010. Secondary metabolic gene clusters: evolutionary toolkits for chemical innovation. *Trends in Genetics,* 26**,** 449-457.

Overmyer, K., Vuorinen, K. & Brosché, M. 2018. Interaction points in plant stress signaling pathways. *Physiologia plantarum,* 162**,** 191-204.

Parker, J. E., Holub, E. B., Frost, L. N., Falk, A., Gunn, N. D. & Daniels, M. J. 1996. Characterization of *eds1*, a mutation in Arabidopsis suppressing resistance to *Peronospora parasitica* specified by several different *RPP* genes. *The Plant Cell,* 8**,** 2033-2046.

Pavgi, M. & Mukhopadhyay, A. 1972. Development of coriander fruit infected by *Protomyces macrosporus* Unger. *Cytologia,* 37**,** 619-627.

Perez-Garcia, L., Diaz-Jimenez, D., Lopez-Esparza, A. & Mora-Montes, H. 2011. Role of cell wall polysaccharides during recognition of *Candida albicans* by the innate immune system. *Glycobiology,* 1**,** 2.

Petre, B., Joly, D. L. & Duplessis, S. 2014. Effector proteins of rust fungi. *Frontiers in plant science,* 5**,** 416.

Petre, B., Saunders, D. G., Sklenar, J., Lorrain, C., Win, J., Duplessis, S. & Kamoun, S. 2015. Candidate effector proteins of the rust pathogen *Melampsora larici-populina* target diverse plant cell compartments. *Molecular Plant-Microbe Interactions,* 28**,** 689-700.

Pétriacq, P., De Bont, L., Tcherkez, G. & Gakière, B. 2013. NAD: not just a pawn on the board of plant-pathogen interactions. *Plant Signaling Behavior,* 8**,** e22477.

Pieterse, C. M., Leon-Reyes, A., Van Der Ent, S. & Van Wees, S. C. 2009. Networking by small-molecule hormones in plant immunity. *Nature chemical biology,* 5**,** 308.

Poppe, S., Dorsheimer, L., Happel, P. & Stukenbrock, E. H. 2015. Rapidly evolving genes are key players in host specialization and virulence of the fungal wheat pathogen *Zymoseptoria tritici* (*Mycosphaerella graminicola*). *PLoS pathogens,* 11**,** e1005055.

Pusztahelyi, T., Holb, I. J. & Pócsi, I. 2015. Secondary metabolites in fungus-plant interactions. *Frontiers in plant science,* 6**,** 573.

Qiu, J. L., Fiil, B. K., Petersen, K., Nielsen, H. B., Botanga, C. J., Thorgrimsen, S., Palma, K., Suarez‐Rodriguez, M. C., Sandbech‐Clausen, S. & Lichota, J. 2008. *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. *The EMBO journal,* 27**,** 2214-2221.

Raacke, I. C., Von Rad, U., Mueller, M. J. & Berger, S. 2006. Yeast increases resistance in *Arabidopsis* against *Pseudomonas syringae* and *Botrytis cinerea* by salicylic acid-dependent as well as -independent mechanisms. *Mol Plant Microbe Interact,* 19**,** 1138-1146.

Raffaele, S., Farrer, R. A., Cano, L. M., Studholme, D. J., Maclean, D., Thines, M., Jiang, R. H., Zody, M. C., Kunjeti, S. G. & Donofrio, N. M. 2010. Genome evolution following host jumps in the Irish potato famine pathogen lineage. *Science,* 330**,** 1540-1543.

Reddy, M. S. & Kramer, C. 1975. A taxonomic revision of the Protomycetales. *Mycotaxon,* 3**,** 1-50.

Rehmany, A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong, M. R., Whisson, S. C., Kamoun, S., Tyler, B. M., Birch, P. R. & Beynon, J. L. 2005. Differential recognition of highly divergent downy mildew avirulence gene alleles by *RPP1* resistance genes from two Arabidopsis lines. *The Plant Cell,* 17**,** 1839-1850.

Rekhter, D., Lüdke, D., Ding, Y., Feussner, K., Zienkiewicz, K., Lipka, V., Wiermer, M., Zhang, Y. & Feussner, I. 2019. Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. 365**,** 498-502.

Ren, D., Liu, Y., Yang, K.-Y., Han, L., Mao, G., Glazebrook, J. & Zhang, S. 2008. A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in *Arabidopsis*. *Proceedings of the National Academy of Sciences,* 105**,** 5638-5643.

Rentel, M. C., Lecourieux, D., Ouaked, F., Usher, S. L., Petersen, L., Okamoto, H., Knight, H., Peck, S. C., Grierson, C. S. & Hirt, H. 2004. OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature,* 427**,** 858.

Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Jiang, C.-Z., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O. & Samaha, R. 2000. *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science,* 290**,** 2105-2110.

Riley, R., Haridas, S., Wolfe, K. H., Lopes, M. R., Hittinger, C. T., Göker, M., Salamov, A. A., Wisecaver, J. H., Long, T. M. & Calvey, C. H. 2016. Comparative genomics of biotechnologically important yeasts. *Proceedings of the National Academy of Sciences,* 113**,** 9882-7.

Robert-Seilaniantz, A., Grant, M. & Jones, J. D. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual review of phytopathology,* 49**,** 317-343.

Robert-Seilaniantz, A., Navarro, L., Bari, R. & Jones, J. D. 2007. Pathological hormone imbalances. *Current opinion in plant biology,* 10**,** 372-379.

Rocha, O., Ansari, K. & Doohan, F. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: a review. *Food Additives & Contaminants,* 22**,** 369-378.

Rogers, E. E., Glazebrook, J. & Ausubel, F. M. 1996. Mode of action of the *Arabidopsis thaliana* phytoalexin camalexin and its role in *Arabidopsis*-pathogen interactions. *Molecular Plant Microbe Interactions,* 9**,** 748-757.

Ryu, C.-M., Farag, M. A., Hu, C.-H., Reddy, M. S., Wei, H.-X., Paré, P. W. & Kloepper, J. W. 2003. Bacterial volatiles promote growth in *Arabidopsis*. *Proceedings of the National Academy of Sciences,* 100**,** 4927-4932.

Saleem, M., Meckes, N., Pervaiz, Z. H. & Traw, M. B. 2017. Microbial interactions in the phyllosphere increase plant performance under herbivore biotic stress. *Frontiers in microbiology,* 8**,** 41.

Santhanam, R., Luu, V. T., Weinhold, A., Goldberg, J., Oh, Y. & Baldwin, I. T. 2015. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proceedings of the National Academy of Sciences,* 112**,** E5013.

Sarris, P. F., Duxbury, Z., Huh, S. U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G. & Saucet, S. B. 2015. A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell,* 161**,** 1089-1100.

Schlaeppi, K. & Mauch, F. 2010. Indolic secondary metabolites protect Arabidopsis from the oomycete pathogen *Phytophthora brassicae*. *Plant Signaling & Behavior,* 5**,** 1099-1101.

Schmitt, I., Crespo, A., Divakar, P., Fankhauser, J., Herman-Sackett, E., Kalb, K., Nelsen, M., Nelson, N., Rivas-Plata, E. & Shimp, A. 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia,* 23**,** 35.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Bolchacova, E., Voigt, K. & Crous, P. W. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proc Natl Acad Sci U S A,* 109**,** 6241-6246.

Schreiber, L., Krimm, U., Knoll, D., Sayed, M., Auling, G. & Kroppenstedt, R. M. 2005. Plant–microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. *New Phytologist,* 166**,** 589-594.

Shan, L., He, P., Li, J., Heese, A., Peck, S. C., Nürnberger, T., Martin, G. B. & Sheen, J. 2008. Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell host & microbe,* 4**,** 17-27.

Sharma, A. & Johri, B. 2003. Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS_9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiological research,* 158**,** 243.

Shibuya, N. & Minami, E. 2001. Oligosaccharide signalling for defence responses in plant. *Physiological Molecular Plant Pathology,* 59**,** 223-233.

Shigenaga, A. M., Berens, M. L., Tsuda, K. & Argueso, C. T. 2017. Towards engineering of hormonal crosstalk in plant immunity. *Current opinion in plant biology,* 38**,** 164-172.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii‐Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H. & Kaku, H. 2010. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *The Plant Journal,* 64**,** 204-214.

Shiu, S.-H. & Bleecker, A. B. 2003. Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. *Plant physiology,* 132**,** 530-543.

Sjamsuridzal, W., Tajiri, Y., Nishida, H., Thuan, T. B., Kawasaki, H., Hirata, A., Yokota, A. & Sugiyama, J. 1997. Evolutionary relationships of membrers of the genera *Taphrina*, *Protomyces*, *Schizosaccharomyces*, and related taxa within the archiascomycetes: Integrated analysis of genotypic and phenotypic characters. *Mycoscience,* 38**,** 267-280.

Sláviková, E., Vadkertiová, R. & Vránová, D. 2009. Yeasts colonizing the leaves of fruit trees. *Annals of microbiology,* 59**,** 419-424.

Spaepen, S. & Vanderleyden, J. 2011. Auxin and plant-microbe interactions. *Cold Spring Harbor perspectives in biology,* 3**,** a001438.

Sperschneider, J., Catanzariti, A.-M., Deboer, K., Petre, B., Gardiner, D. M., Singh, K. B., Dodds, P. N. & Taylor, J. M. 2017. LOCALIZER: subcellular localization prediction of both plant and effector proteins in the plant cell. *Scientific reports,* 7**,** 44598.

Sperschneider, J., Dodds, P. N., Gardiner, D. M., Singh, K. B. & Taylor, J. M. 2018a. Improved prediction of fungal effector proteins from secretomes with EffectorP 2.0. *Molecular plant pathology,* 19**,** 2094-2110.

Sperschneider, J., Dodds, P. N., Singh, K. B. & Taylor, J. M. 2018b. ApoplastP: prediction of effectors and plant proteins in the apoplast using machine learning. *New Phytologist,* 217**,** 1764-1778.

Sperschneider, J., Gardiner, D. M., Dodds, P. N., Tini, F., Covarelli, L., Singh, K. B., Manners, J. M. & Taylor, J. M. 2016. EffectorP: predicting fungal effector proteins from secretomes using machine learning. *New Phytologist,* 210**,** 743-761.

Stergiopoulos, I. & De Wit, P. J. 2009. Fungal effector proteins. *Annual review of phytopathology,* 47**,** 233-263.

Stielow, J. B., Levesque, C. A., Seifert, K. A., Meyer, W., Iriny, L., Smits, D., Renfurm, R., Verkley, G., Groenewald, M. & Chaduli, D. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia: Molecular Phylogeny Evolution of Fungi,* 35**,** 242.

Su, J., Xu, J. & Zhang, S. 2015. RACK1, scaffolding a heterotrimeric G protein and a MAPK cascade. *Trends in plant science,* 20**,** 405-407.

Sugiyama, J., Hosaka, K. & Suh, S.-O. 2006. Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. *Mycologia,* 98**,** 996-1005.

Sugiyama, J., Nagahama, T. & Nishida, H. 1996. Fungal diversity and phylogeny with emphasis on 18S ribosomal DNA sequence divergence. *In:* RR, C., U, S. & K, O. (eds.) *Microbial diversity in time and space.* Springer.

Suh, M.-J., Fedorova, N. D., Cagas, S. E., Hastings, S., Fleischmann, R. D., Peterson, S. N., Perlin, D. S., Nierman, W. C., Pieper, R. & Momany, M. 2012. Development stage-specific proteomic profiling uncovers small, lineage specific proteins most abundant in the *Aspergillus Fumigatus* conidial proteome. *Proteome Science,* 10**,** 30.

Sy, A., Timmers, A. C., Knief, C. & Vorholt, J. A. 2005. Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. *Appl. Environ. Microbiol.,* 71**,** 7245-7252.

Tada, Y., Spoel, S. H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J. & Dong, X. 2008. Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins. *Science,* 321**,** 952-956.

Takahashi, F., Yoshida, R., Ichimura, K., Mizoguchi, T., Seo, S., Yonezawa, M., Maruyama, K., Yamaguchi-Shinozaki, K. & Shinozaki, K. 2007. The mitogen-activated protein kinase cascade MKK3–MPK6 is an

important part of the jasmonate signal transduction pathway in *Arabidopsis*. *The Plant Cell,* 19**,** 805-818.

Teige, M., Scheikl, E., Eulgem, T., Dóczi, R., Ichimura, K., Shinozaki, K., Dangl, J. L. & Hirt, H. 2004. The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. *Molecular cell,* 15**,** 141-152.

Thomma, B. P., Nelissen, I., Eggermont, K. & Broekaert, W. F. 1999. Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. *The Plant Journal,* 19**,** 163-171.

Thomma, B. P., Nürnberger, T. & Joosten, M. H. 2011. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *The plant cell,* 23**,** 4-15.

Tian, W., Hou, C., Ren, Z., Wang, C., Zhao, F., Dahlbeck, D., Hu, S., Zhang, L., Niu, Q., Li, L., Staskawicz, B. J. & Luan, S. 2019. A calmodulin-gated calcium channel links pathogen patterns to plant immunity. *Nature*.

Tubaki, K. 1957. Biological and cultural studies of three species of *Protomyces*. *Mycologia,* 49**,** 44-54.

Tukey Jr, H. 1970. The leaching of substances from plants. *Annual review of plant physiology,* 21**,** 305-324.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z. & Marhold, K. 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books*.

Tuzet, A., Rahantaniaina, M.-S. & Noctor, G. 2018. Analyzing the function of catalase and the ascorbate–glutathione pathway in H2O2 processing: insights from an experimentally constrained kinetic model. *Antioxidants and Redox Signaling*.

Tyagi, S., Mulla, S. I., Lee, K.-J., Chae, J.-C. & Shukla, P. 2018. VOCs-mediated hormonal signaling and crosstalk with plant growth promoting microbes. *Critical reviews in biotechnology,* 38**,** 1277-1296.

Unger, F. 1833. Die Exantheme der Pflanzen. *Gerold, Wien***,** 421.

Vacher, C., Hampe, A., Porté, A. J., Sauer, U., Compant, S. & Morris, C. E. 2016. The phyllosphere: microbial jungle at the plant–climate interface. *Annual Review of Ecology, Evolution and Systematics,* 47**,** 1-24.

Vadassery, J., Ritter, C., Venus, Y., Camehl, I., Varma, A., Shahollari, B., Novák, O., Strnad, M., Ludwig-Müller, J. & Oelmüller, R. 2008. The role of auxins and cytokinins in the mutualistic interaction between *Arabidopsis* and *Piriformospora indica*. *Molecular Plant Microbe Interactions,* 21**,** 1371-1383.

Valadon, L., Manners, J. & Myers, A. 1962. Studies on the life-history and taxonomic position of *Protomyces inundatus* Dangeard. *Transactions of the British Mycological Society,* 45**,** 573-86.

Van Den Burg, H. A., Harrison, S. J., Joosten, M. H., Vervoort, J. & De Wit, P. J. 2006. *Cladosporium fulvum* Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. *Molecular Plant-Microbe Interactions,* 19**,** 1420-1430.

Van Der Burgh, A. M. & Joosten, M. H. 2019. Plant immunity: thinking outside and inside the box. *Trends in Plant Science*.

Vanetten, H. D., Mansfield, J. W., Bailey, J. A. & Farmer, E. E. 1994. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". *The Plant Cell,* 6**,** 1191.

Wang, C., Zhou, M., Zhang, X., Yao, J., Zhang, Y. & Mou, Z. 2017. A lectin receptor kinase as a potential sensor for extracellular nicotinamide adenine dinucleotide in *Arabidopsis thaliana*. *eLife,* 6**,** e25474.

Wang, H., Wang, H., Shao, H. & Tang, X. 2016. Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. *Frontiers in plant science,* 7**,** 67.

Wang, J., Hu, M., Wang, J., Qi, J., Han, Z., Wang, G., Qi, Y., Wang, H.-W., Zhou, J.-M. & Chai, J. 2019a. Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science,* 364**,** eaav5870.

Wang, J., Wang, J., Hu, M., Wu, S., Qi, J., Wang, G., Han, Z., Qi, Y., Gao, N. & Wang, H.-W. 2019b. Ligand-triggered allosteric ADP release primes a plant NLR complex. *Science,* 364**,** eaav5868.

Wang, Y., Schuck, S., Wu, J., Yang, P., Doering, A.-C., Zeier, J. & Tsuda, K. 2018. A MPK3/6-WRKY33-ALD1-Pipecolic acid regulatory loop contributes to systemic acquired resistance. *The Plant Cell*.

Waszczak, C., Carmody, M. & Kangasjärvi, J. 2018. Reactive oxygen species in plant signaling. *Annual review of plant biology,* 69**,** 209-236.

Weber, T., Blin, K., Duddela, S., Krug, D., Kim, H. U., Bruccoleri, R., Lee, S. Y., Fischbach, M. A., Müller, R. & Wohlleben, W. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic acids research,* 43**,** W237-W243.

Wiegmann, B. M., Trautwein, M. D., Kim, J.-W., Cassel, B. K., Bertone, M. A., Winterton, S. L. & Yeates, D. K. 2009. Single-copy nuclear genes resolve the phylogeny of the holometabolous insects. *BMC biology,* 7**,** 34.

Withers, J. & Dong, X. 2016. Posttranslational modifications of NPR1: a single protein playing multiple roles in plant immunity and physiology. *PLoS pathogens,* 12**,** e1005707.

Withers, J. & Dong, X. 2017. Post-translational regulation of plant immunity. *Current opinion in plant biology,* 38**,** 124-132.

Vlot, A. C., Dempsey, D. M. A. & Klessig, D. F. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual review of phytopathology,* 47**,** 177-206.

Von Arx, J. & Weijman, A. 1979. Conidiation and carbohydrate composition in some *Candida* and *Torulopsis* species. *Antonie van Leeuwenhoek,* 45**,** 547-555.

Vorholt, J. A. 2012. Microbial life in the phyllosphere. *Nat Rev Micro,* 10**,** 828-840.

Wrzaczek, M., Brosché, M. & Kangasjärvi, J. 2013. ROS signaling loops—production, perception, regulation. *Current opinion in plant biology,* 16**,** 575-582.

Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., De Luca, V. & Després, C. 2012. The *Arabidopsis* NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell reports,* 1**,** 639-647.

Xiang, T., Zong, N., Zhang, J., Chen, J., Chen, M. & Zhou, J.-M. 2011. BAK1 is not a target of the *Pseudomonas syringae* effector AvrPto. *Molecular plant-microbe interactions,* 24**,** 100-107.

Xu, G., Greene, G. H., Yoo, H., Liu, L., Marqués, J., Motley, J. & Dong, X. 2017. Global translational reprogramming is a fundamental layer of immune regulation in plants. *Nature,* 545**,** 487.

Xu, J. & Zhang, S. 2015. Mitogen-activated protein kinase cascades in signaling plant growth and development. *Trends in plant science,* 20**,** 56-64.

Yang, C.-H., Crowley, D. E., Borneman, J. & Keen, N. T. 2001. Microbial phyllosphere populations are more complex than previously realized. *Proceedings of the National Academy of Sciences,* 98**,** 3889-3894.

Yin, C., Ramachandran, S. R., Zhai, Y., Bu, C., Pappu, H. R. & Hulbert, S. H. 2019. A novel fungal effector from *Puccinia graminis* suppressing RNA silencing and plant defense responses. *New Phytologist*.

Yoo, S.-D., Cho, Y.-H., Tena, G., Xiong, Y. & Sheen, J. 2008. Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling. *Nature,* 451**,** 789.

Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X. & Chen, S. 2010. Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell host & microbe,* 7**,** 290-301.

Zhang, J., Shao, F., Li, Y., Cui, H., Chen, L., Li, H., Zou, Y., Long, C., Lan, L. & Chai, J. 2007. A *Pseudomonas syringae* effector inactivates MAPKs to suppress PAMP-induced immunity in plants. *Cell host & microbe,* 1**,** 175-185.

Zhang, M., Chiang, Y.-H., Toruño, T. Y., Lee, D., Ma, M., Liang, X., Lal, N. K., Lemos, M., Lu, Y.-J. & Ma, S. 2018. The MAP4 Kinase SIK1 ensures robust extracellular ROS burst and antibacterial immunity in plants. *Cell host & microbe,* 24**,** 379-391. e5.

Zhang, Y., Tessaro, M. J., Lassner, M. & Li, X. 2003. Knockout analysis of Arabidopsis transcription factors *TGA2*, *TGA5*, and *TGA6* reveals their redundant and essential roles in systemic acquired resistance. *The Plant Cell,* 15**,** 2647-2653.

Zhang, Z., Wu, Y., Gao, M., Zhang, J., Kong, Q., Liu, Y., Ba, H., Zhou, J. & Zhang, Y. 2012. Disruption of PAMP-induced MAP kinase cascade by a *Pseudomonas syringae* effector activates plant immunity mediated by the NB-LRR protein SUMM2. *Cell host & microbe,* 11**,** 253-263.

Zhao, Z., Liu, H., Wang, C. & Xu, J.-R. 2013. Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. *BMC genomics,* 14**,** 274.

Zipfel, C. & Felix, G. 2005. Plants and animals: a different taste for microbes? *Current opinion in plant biology,* 8**,** 353-360.

Ziv, C., Zhao, Z., Gao, Y. G. & Xia, Y. 2018. Multifunctional roles of plant cuticle during plant-pathogen interactions. *Frontiers in plant science,* 9**,** 1088.